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The application was originally filed in English.

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IMMEDIATE RELEASE PHARMACEUTICAL FORMULATION

This invention relates to a novel immediate release pharmaceutical formulation that provides for the delivery of particular pharmaceuticals, to the manufacture of such a formulation, and to the use of such a formulation in the treatment or prevention of thrombosis.

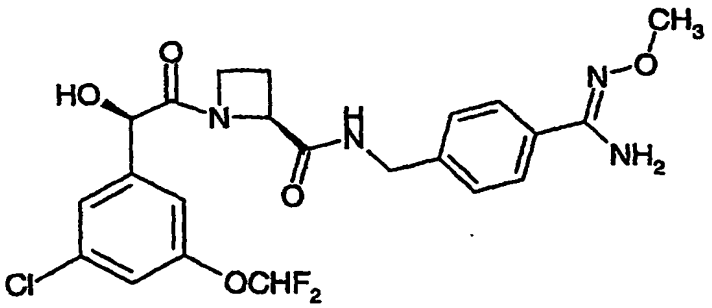
It is often desirable to formulate pharmaceutically active compounds for immediate release following oral and/or parenteral administration with a view to providing a sufficient concentration of drug in plasma within the time-frame required to give rise to a desired therapeutic response.

Immediate release may be particularly desirable in cases where, for example, a rapid therapeutic response is required (for example in the treatment of acute problems), or, in the case of parenteral administration, when peroral delivery to the gastrointestinal tract is incapable of providing sufficient systemic uptake within the required time-frame.

In the case of the treatment or prophylaxis of thrombosis, immediate release formulations may be necessary to ensure that a sufficient amount of drug is provided in plasma within a relatively short period of time to enable quick onset of action. Immediate release formulations are also typically simpler to develop than modified release formulations, and may also provide more flexibility in relation to the variation of doses that are to be administered to patients. Immediate release formulations are superior when multiple doses are not required and where it is not necessary to keep the plasma concentration at a constant level for an extended time.

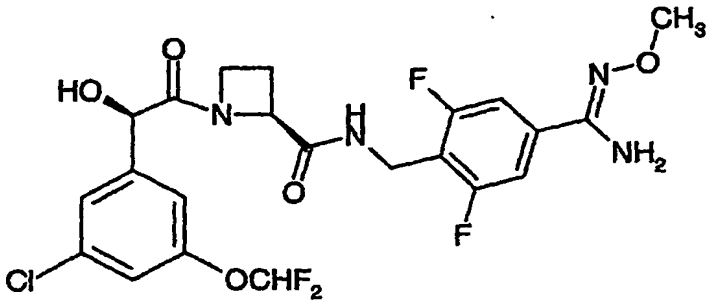
Unpublished international patent application No. PCT/SE01/02657 discloses a number of compounds that are, or are metabolised to compounds which are, competitive inhibitors of trypsin-like proteases, such as thrombin. The following three compounds are amongst those that are specifically disclosed:

(a) Ph(3-Cl)(5-$OCHF_2$)-(*R*)CH(OH)C(O)-(*S*)Aze-Pab(OMe):



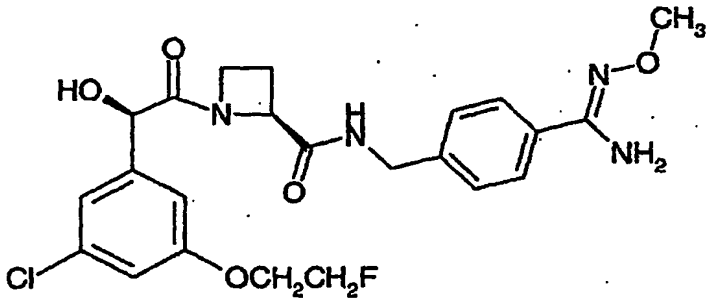
which compound is referred to hereinafter as Compound A;

(b) Ph(3-Cl)(5-$OCHF_2$)-(*R*)CH(OH)C(O)-(*S*)Aze-Pab(2,6-diF)(OMe):



which compound is referred to hereinafter as Compound B; and

(c) Ph(3-Cl)(5-OCH_2CH_2F)-(*R*)CH(OH)C(O)-(*S*)Aze-Pab(OMe):



which compound is referred to hereinafter as Compound C.

The methoxyamidine Compounds A, B and C are metabolised following oral and/or parenteral administration to the corresponding free amidine compounds, which latter compounds have been found to be potent inhibitors of thrombin. Thus:

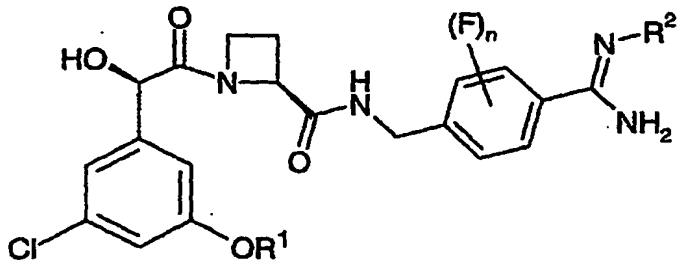
- Compound A is metabolized to Ph(3-Cl)(5-$OCHF_2$)-(*R*)CH(OH)C(O)-(*S*)Aze-Pab (which compound is referred to hereinafter as Compound D) via a prodrug intermediate

Ph(3-Cl)(5-$OCHF_2$)-(*R*)CH(OH)C(O)-(*S*)Aze-Pab(OH) (which compound is referred to hereinafter as Compound G);

- Compound B is metabolized to Ph(3-Cl)(5-$OCHF_2$)-(*R*)CH(OH)C(O)-(*S*)Aze-Pab(2,6-diF) (which compound is referred to hereinafter as Compound E) via a prodrug intermediate Ph(3-Cl)(5-$OCHF_2$)-(*R*)CH(OH)C(O)-(*S*)Aze-Pab(2,6-diF)(OH) (which compound is referred to hereinafter as Compound H); and,
- Compound C is metabolized to Ph(3-Cl)(5-OCH_2CH_2F)-(*R*)CH(OH)C(O)-(*S*)Aze-Pab (which compound is referred to hereinafter as Compound F) via a prodrug intermediate Ph(3-Cl)(5-OCH_2CH_2F)-(*R*)CH(OH)C(O)-(*S*)Aze-Pab(OH) (which compound is referred to hereinafter as Compound J).

Processes for the synthesis of Compounds A, B, C, D, E, F, G and J are described in Examples 12, 40, 22, 3, 39, 21, 2 and 31 (respectively) of international patent application No. PCT/SE01/02657. An immediate release formulation of these compounds, or their metabolites has yet to be described in the literature. We have found that the compounds of formula (I) and their salts can be formulated as immediate release pharmaceutical formulations which are easy to administer, for example by oral or parenteral administration.

According to a first aspect of the invention, there is provided an immediate release pharmaceutical formulation comprising, as active ingredient, a compound of formula (I):



(I)

wherein

R^1 represents C_{1-2} alkyl substituted by one or more fluoro substituents;

R^2 represents hydrogen, hydroxy, methoxy or ethoxy; and

n represents 0, 1 or 2;

or a pharmaceutically acceptable salt thereof; and a pharmaceutically acceptable diluent or carrier;

provided that the formulation does not solely contain:

- a solution of one active ingredient and water;
- a solution of one active ingredient and dimethylsulphoxide; or,
- a solution of one active ingredient in a mixture of ethanol : PEG 660 12-hydroxy stearate : water 5:5:90;

which formulations are referred to hereinafter as "the formulations of the invention".

PEG 660 12-hydroxy stearate is a non-ionic surfactant and is better known as Solutol K ™.

According to a second aspect of the present invention there is provided Compound H, Ph(3-Cl)(5-$OCHF_2$)-(*R*)CH(OH)C(O)-(*S*)Aze-Pab(2,6-diF)(OH) which can be prepared by methods similar to those described below for the preparation of Compounds G and J.

The compounds of formula (I) may be in the form of a solvate, a hydrate, a mixed solvate/hydrate or, preferably, an ansolvate, such as an anhydrate. Solvates may be of one or more organic solvents, such as lower (for example C_{1-4}) alkyl alcohols (for example methanol, ethanol or *iso*-propanol), ketones (such as acetone), esters (such as ethyl acetate) or mixtures thereof.

In one particular aspect of the invention R^1 is CHF_2 or CH_2CH_2F.

The variable n is preferably 0 or 2.

More preferred compounds of formula (I) include those in which n represents 0, or those in which n represents 2, so providing two fluoro atoms located at the 2- and 6-positions (that is the two *ortho*-positions relative to the point of attachment of the benzene ring to the $-NH-CH_2-$ group).

The compound of formula (I) is especially Compound A, Compound B or Compound C.

Preferred salts of the compounds of formula (I) are acid addition salts. Acid addition salts include inorganic acid addition salts, such as those of sulphuric acid, nitric acid, phosphoric acid and hydrohalic acids, such as hydrobromic acid and hydrochloric acid. More preferred acid addition salts include those of organic acids, such as those of

dimethylphosphoric acid; saccharinic acid; cyclohexylsulfamic acid; those of carboxylic acids (such as maleic acid, fumaric acid, aspartic acid, succinic acid, malonic acid, acetic acid, benzoic acid, terephthalic acid, hippuric acid, 1-hydroxy-2-naphthoic acid, pamoic acid, hydroxybenzoic acid and the like); those of hydroxy acids (such as salicylic acid, tartaric acid, citric acid, malic acid (including L-(-)-malic acid and, D,L-malic acid), gluconic acid (including D-gluconic acid), glycolic acid, ascorbic acid, lactic acid and the like); those of amino acids (such as glutamic acid (including D-glutamic, L-glutamic, and D,L-glutamic, acids), arginine (including L-arginine), lysine (including L-lysine and L-lysine hydrochloride), glycine and the like); and, particularly, those of sulfonic acids, (such as 1,2-ethanedisulfonic acid, camphorsulfonic acids (including 1S-(+)-10-camphorsulfonic acid and (+/-)-camphorsulfonic acids), ethanesulfonic acid, a propanesulfonic acid (including *n*-propanesulfonic acid), a butanesulfonic acid, a pentanesulfonic acid, a toluenesulfonic acid, methanesulfonic acid, p-xylenesulfonic acid, 2-mesitylenesulfonic acid, naphthalenesulfonic acids (including 1,5-naphthalenesulfonic acid and naphthalenesulfonic acid), benzenesulfonic acid, hydroxybenzenesulfonic acids, 2-hydroxyethanesulfonic acid, 3-hydroxyethanesulfonic acid and the like).

Particularly preferred salts include those of C_{1-6} (for example C_{1-4}) alkanesulfonic acids, such as ethanesulfonic acid (esylate) and propanesulfonic acid (for example *n*-propanesulfonic acid) and optionally substituted (for example with one or more C_{1-2} alkyl groups) arylsulfonic acids, such as benzenesulfonic acid (besylate).

Suitable stoichiometric ratios of acid to free base are in the range 0.25:1.5 to 3.0:1, such as 0.45:1.25 to 1.25:1, including 0.50:1 to 1:1.

According to a further aspect of the invention there is provided formulation comprising a compound of formula (I) in substantially crystalline form.

Although we have found that it is possible to produce compounds of the invention in forms which are greater than 80% crystalline, by "substantially crystalline" we include greater than 20%, preferably greater than 30%, and more preferably greater than 40% (e.g. greater than any of 50, 60, 70, 80 or 90%) crystalline. The degree (%) of crystallinity may be determined by the skilled person using X-ray powder diffraction (XRPD). Other

techniques, such as solid state NMR, FT-IR, Raman spectroscopy, differential scanning calorimetry (DSC) and microcalorimetry, may also be used.

Preferred compounds of formula (I) that may be prepared in crystalline form include salts of C_{1-6} (for example C_{2-6}, such as C_{2-4}) alkanesulfonic acids, such as ethanesulfonic acid, propanesulfonic acid (for example *n*-propanesufonic acid) and optionally substituted arylsulfonic acids, such as benzenesulfonic acid.

The term "immediate release" pharmaceutical formulation includes any formulation in which the rate of release of drug from the formulation and/or the absorption of drug, is neither appreciably, nor intentionally, retarded by galenic manipulations. In the present case, immediate release may be provided for by way of an appropriate pharmaceutically acceptable diluent or carrier, which diluent or carrier does not prolong, to an appreciable extent, the rate of drug release and/or absorption. Thus, the term excludes formulations which are adapted to provide for "modified", "controlled", "sustained", "prolonged", "extended" or "delayed" release of drug.

In this context, the term "release" includes the provision (or presentation) of drug from the formulation to the gastrointestinal tract, to body tissues and/or into systemic circulation.

Thus, formulations of the invention may release at least 70% (preferably 80%) of active ingredient within 4 hours, such as within 3 hours, preferably 2 hours, more preferably within 1.5 hours, and especially within an hour (such as within 30 minutes), of administration, whether this be oral or parenteral.

The formulations of the invention may be formulated in accordance with a variety of known techniques, for example as described by M. E. Aulton in "*Pharmaceutics: The Science of Dosage Form Design*" (1988) (Churchill Livingstone), the relevant disclosures in which document are hereby incorporated by reference.

Formulations of the invention may be, or may be adapted in accordance with standard techniques to be, suitable for peroral administration, for example in the form of an immediate release tablet, an immediate release capsule or as a liquid dosage form, comprising active ingredient. These formulation types are well known to the skilled person and may be prepared in accordance with techniques known in the art.

Suitable diluents/carriers (which may also be termed "fillers") for use in peroral formulations of the invention, for example those in the form of immediate release tablets, include monobasic calcium phosphate, dibasic calcium phosphate (including dibasic calcium phosphate dihydrate and dibasic calcium phosphate anhydrate), tribasic calcium phosphate, lactose, microcrystalline cellulose, silicified microcrystalline cellulose, mannitol, sorbitol, starch (such as maize, potato or rice), glucose, calcium lactate, calcium carbonate and the like. Preferred diluents/carriers include dibasic calcium phosphate and microcrystalline cellulose, which may be used alone or in combination with another diluent/carrier such as mannitol.

A formulation of the invention in the form of an immediate release tablet may comprise one or more excipients to improve the physical and/or chemical properties of the final composition, and/or to facilitate the process of manufacture. Such excipients are conventional in the formulation of immediate release formulations for peroral drug delivery, and include one or more of the following: one or more lubricants (such as magnesium stearate, stearic acid, calcium stearate, stearyl alcohol or, preferably, sodium stearyl fumarate); a glidant (such as talc or a colloidal silica); one or more binders (such as polyvinylpyrrolidone, microcrystalline cellulose, a polyethylene glycol (PEG), a polyethylene oxide, a hydroxypropylmethylcellulose (HPMC) of a low molecular weight, a methylcellulose (MC) of a low molecular weight, a hydroxypropylcellulose (HPC) of a low molecular weight, a hydroxyethylcellulose (HEC) of a low molecular weight, a starch (such as maize, potato or rice) or a sodium carboxymethyl cellulose of a low molecular weight; (preferred binders are polyvinylpyrrolidone or a HPMC of a low molecular weight); one or more pH controlling agents (such as an organic acid (for example citric acid) or an alkali metal (for example sodium) salt thereof, an oxide of magnesium, an alkali or alkaline earth metal (for example sodium, calcium or potassium) sulphate, metabisulphate, propionate or sorbate); one or more disintegrant (for example sodium starch glycollate, a crosslinked polyvinylpyrrolidone, a crosslinked sodium carboxymethyl cellulose, a starch (such as maize, potato or rice) or an alginate); a colourant, a flavouring, a tonicity-modifying agent, a coating agent or a preservative.

It will be appreciated that some of the above mentioned excipients which may be present in a final immediate release oral (for example tablet) formulation of the invention may have more than one of the above-stated functions.

In a further aspect of the invention a formulation of the invention is adapted to be suitable for parenteral administration. The term "parenteral" includes any mode of administration that does not comprise peroral administration to the gastrointestinal tract and includes administration subcutaneously, intravenously, intraarterially, transdermally, intranasally, intrabuccally, intracutaneously, intramuscularly, intralipomateously, intraperitoneally, rectally, sublingually, topically, by inhalation, or by any other parenteral route.

Suitable formulations of the invention that are to be administered parenterally include those in which a compound of formula (I) or a pharmaceutically acceptable salt thereof is presented together with an aqueous carrier, such as water.

A formulation of the present invention comprising an aqueous carrier may further comprise one or more excipients, such as an antimicrobial preservative; a tonicity modifier (for example sodium chloride, mannitol or glucose); a pH adjusting agent (for example a common inorganic acid or base, including hydrochloric acid or sodium hydroxide); a pH controlling agents (that is, a buffer; for example tartaric acid, acetic acid or citric acid); a surfactant (for example Solutol™); a solubiliser which serves to help solubilise the active ingredient (for example ethanol, a polyethylene glycol or hydroxypropyl-β-cyclodextrin); or an antioxidant.

Parenteral formulations may be in the form of suspensions of active ingredient in association with an aqueous solvent or, more preferably aqueous solutions (that is, solutions of active compound including water as a solvent). In this context, the term "aqueous solution" includes formulations in which at least 99% of active ingredient is in solution at above 5°C and atmospheric pressure, and the term "suspension" means that more than 1% of active ingredient is not in solution under such conditions.

The number of excipients employed in the peroral and parenteral formulations of the invention depends upon many factors, such as the nature and amount of active ingredient present, and the amount of diluent/carrier (aqueous solvent or otherwise) that is included.

In another aspect the present invention provides a parenteral formulation comprising a compound of formula (I), or a pharmaceutically acceptable salt thereof, water and at least one additional agents. The additional agents include:

i. polyethylene glycol (PEG) and optionally also ethanol and/or tartaric acid and/or hydrochloric acid; or

ii. sodium chloride (which will be dissolved in the formulation), and optionally also ethanol; or

iii. hydrochloric acid and/or sodium hydroxide to bring the pH to a suitable value (preferably in the range 3-8 for a compound of formula (I) wherein R^2 is hydrogen, such as Compound D, E or F; or preferably in the range 3.5-8 for a compound of formula (I) wherein R^2 is methoxy or ethoxy, such as Compound A, B or C); or

iv. DMA (dimethyl acetamide) and optionally also a medium chain triglyceride (such as miglyol); or

v. a β-cyclodextrin (such as hydroxypropyl-β-cyclodextrin);

vi. a tonicity modifier such as sodium chloride and/or mannitol.

In a further aspect the present invention provides an injectable solution comprising a compound of formula (I), or a pharmaceutically acceptable salt thereof, (preferably Compound D, E or F) water and at least one additional agents as recited in (i) to (vi) above.

In another aspect the invention provides an aqueous formulation of a compound of formula (I) (such as Compound D, E or F) comprising a solubilising agent such as a polyethylene glycol, β-cyclodextrin (such as hydroxypropyl-β-cyclodextrin), sorbitol or ethanol.

In a further aspect the present invention provides a parenteral formulation comprising a compound of formula (I) and ethanol. This formulation can further comprise a medium chain triglyceride (such as miglyol).

In a still further aspect the present invention provides a parenteral formulation comprising a compound of formula (I) and DMA. This formulation can further comprise a medium chain triglyceride (such as miglyol).

In another aspect the compound of formula (I) is crystalline (especially a salt of Compound A; preferably a C_{1-6} (for example C_{2-6}, such as C_{2-4}) alkanesulfonic acid salt,

such as ethanesulfonic acid, propanesulfonic acid (for example *n*-propanesufonic acid) or an optionally substituted arylsulfonic acid salt, such as benzenesulfonic acid salt).

In yet another aspect the formulation of the present invention is in a solid dosage form wherein R^2 is hydroxy, methoxy or ethoxy (preferably methoxy) (the compound of formula (I) is especially Compound A, Compound B or Compound C).

In yet another aspect the present invention provides a parenteral formulation (especially a water-based, injectable solution) comprising a compound of formula (I) in free base form.

In a further aspect the present invention provides a parenteral formulation comprising a compound of formula (I) in free base form wherein R^2 is hydrogen.

In a still further aspect the present invention provides a solid formulation comprising microcrystalline cellulose and polyvinyl pyrrolidone (PVP); or comprising microcrystalline cellulose and sodium starch glycollate.

Formulations of the invention, such as parenteral formulations, comprising salts may be prepared by addition of diluent/carrier to the appropriate pre-prepared salt.

Compositions including active ingredient may also be provided in solid form suitable for use in the preparation of a formulation of the invention (for example a solution, such as an aqueous solution, for example for parenteral adminstration) *ex tempore*. Such compositions may be in the form of a solid comprising active ingredient, optionally in the presence of one or more further excipients as hereinbefore defined and, optionally, up to 10% (w/w) of diluent and/or carrier as hereinbefore defined, which compositions are hereinafter referred to as "the solid compositions of the invention".

Solid compositions of the invention may be made by removal of diluent/carrier (for example solvent) from a formulation of the invention, or a concentrated formulation of the invention, which may for example be in the form of a solution, such as an aqueous solution.

In another aspect the present invention provides an orally administerable, immediate release formulation comprising a compound of formula (I), or a salt thereof, a carrier (such as microcrystalline cellulose), a disintegrant (such as sodium starch glycollate), a binder (such as polyvinyl pyrrolidone) and a lubricant (such as sodium stearyl

fumarate). Such a formulation may also comprise an additional carrier (or filler) such as mannitol.

Formulations of the invention that are in the form of immediate release tablets may be prepared by bringing active ingredient into association with diluent/carrier using standard techniques, and using standard equipment, known to the skilled person, including wet or dry granulation, direct compression/compaction, drying, milling, mixing, tableting and coating, as well as combinations of these processes, for example as described hereinafter.

There is thus provided a process for the formation of a solid composition suitable for use in the preparation of a formulation of the invention (for example a solution, such as an aqueous solution) *ex tempore*, which process comprises removal of diluent/carrier (for example solvent) from a formulation of the invention, or a concentrated formulation of the invention.

Solvent may be removed by way of a variety of techniques known to those skilled in the art, for example evaporation (under reduced pressure or otherwise), freeze-drying, or any solvent removal (drying) process that removes solvent (such as water) while maintaining the integrity of the active ingredient. An example of drying is freeze-drying.

Thus according to a further aspect of the invention there is provided a freeze-dried (lyophilised) solid composition of the invention.

In the preparation of solid compositions of the invention, the skilled person will appreciate that appropriate additional excipients may be added at a suitable stage prior to removal of diluent/carrier. For example, in the case of aqueous solutions, pH may be controlled and/or adjusted as hereinbefore described. Furthermore, an appropriate additional excipient may be added with a view to aiding the formation of a solid composition of the invention during the process of diluent/carrier removal (for example mannitol, sucrose, glucose, mannose or trehalose).

A solid composition of a compound of formula (I) or a salt thereof, thus includes a composition in which the solvent (for example water) content, other than a solvent of crystallization, is no more than 10%, such as less than 2% unbound solvent, such as water.

Formulations of the invention may be sterilised, for example by sterile filtration or autoclavation, and/or filled into primary packages, such as vials, cartridges and pre-filled syringes. Such processing steps may also take place prior to drying to form a solid composition of the invention.

Before administration, the dried solid composition may be reconstituted and/or diluted in, for instance, water, physiological saline, glucose solution or any other suitable solution.

The amount of diluent/carrier in an oral (for example immediate release tablet) formulation of the invention depends upon many factors, such as the nature and amount of the active ingredient that is employed and the nature, and amounts, of any other constituents (for example further excipients) that are present in the formulation, but is typically up to 40% (w/w), preferably up to 30%, more preferably up to 20%, and particularly up to 10% (w/w) of the final composition. The amount of additional excipients in such an oral formulation of the invention also depends upon factors, such as the nature and amount of the active ingredient that is employed, as well as the nature, and amounts, of any other constituents (for example diluents/carriers and/or other further excipients) that are present in the formulation, but, for lubricants and glidants is typically up to 5% (w/w), and for binders and disintegrants is typically up to 10% (w/w) of the final composition.

The formulations of the invention are administered to mammalian patients (including humans), and, for compounds of formula (I) wherein R^2 is not hydrogen, are thereafter metabolised in the body to form compounds of formula (I) wherein R^2 is hydrogen that are pharmacologically active.

According to a further aspect of the invention there is thus provided a formulation of the invention for use as a pharmaceutical.

In particular, the compounds of formula (I) are, or are metabolised following administration to form, potent inhibitors of thrombin, for example as may be demonstrated in the tests described in *inter alia* international patent application No. PCT/SE01/02657, as well as international patent applications WO 02/14270, WO 01/87879 and WO 00/42059, the relevant disclosures in which documents are hereby incorporated by reference.

By "prodrug of a thrombin inhibitor", we include compounds that are metabolised following administration and form a thrombin inhibitor, in an experimentally-detectable amount, following administration.

The formulations of the invention are thus expected to be useful in those conditions where inhibition of thrombin is required, and/or conditions where anticoagulant therapy is indicated, including the following:

The treatment and/or prophylaxis of thrombosis and hypercoagulability in blood and/or tissues of animals including man. It is known that hypercoagulability may lead to thrombo-embolic diseases. Conditions associated with hypercoagulability and thrombo-embolic diseases which may be mentioned include inherited or acquired activated protein C resistance, such as the factor V-mutation (factor V Leiden), and inherited or acquired deficiencies in antithrombin III, protein C, protein S, heparin cofactor II. Other conditions known to be associated with hypercoagulability and thrombo-embolic disease include circulating antiphospholipid antibodies (Lupus anticoagulant), homocysteinemi, heparin induced thrombocytopenia and defects in fibrinolysis, as well as coagulation syndromes (for example disseminated intravascular coagulation (DIC)) and vascular injury in general (for example due to surgery).

The treatment of conditions where there is an undesirable excess of thrombin without signs of hypercoagulability, for example in neurodegenerative diseases such as Alzheimer's disease.

Particular disease states which may be mentioned include the therapeutic and/or prophylactic treatment of venous thrombosis (for example DVT) and pulmonary embolism, arterial thrombosis (e.g. in myocardial infarction, unstable angina, thrombosis-based stroke and peripheral arterial thrombosis), and systemic embolism usually from the atrium during atrial fibrillation (for example non-valvular atrial fibrillation) or from the left ventricle after transmural myocardial infarction, or caused by congestive heart failure; prophylaxis of re-occlusion (that is thrombosis) after thrombolysis, percutaneous trans-luminal angioplasty (PTA) and coronary bypass operations; the prevention of re-thrombosis after microsurgery and vascular surgery in general.

Further indications include the therapeutic and/or prophylactic treatment of disseminated intravascular coagulation caused by bacteria, multiple trauma, intoxication or any other mechanism; anticoagulant treatment when blood is in contact with foreign surfaces in the body such as vascular grafts, vascular stents, vascular catheters, mechanical and biological prosthetic valves or any other medical device; and anticoagulant treatment when blood is in contact with medical devices outside the body such as during cardiovascular surgery using a heart-lung machine or in haemodialysis; the therapeutic and/or prophylactic treatment of idiopathic and adult respiratory distress syndrome, pulmonary fibrosis following treatment with radiation or chemotherapy, septic shock, septicemia, inflammatory responses, which include, but are not limited to, edema, acute or chronic atherosclerosis such as coronary arterial disease and the formation of atherosclerotic plaques, cerebral arterial disease, cerebral infarction, cerebral thrombosis, cerebral embolism, peripheral arterial disease, ischaemia, angina (including unstable angina), reperfusion damage, restenosis after percutaneous trans-luminal angioplasty (PTA) and coronary artery bypass surgery.

The formulation of the present invention may also comprise any antithrombotic agent(s) with a different mechanism of action to that of the compounds of formula (I), such as one or more of the following: the antiplatelet agents acetylsalicylic acid, ticlopidine and clopidogrel; thromboxane receptor and/or synthetase inhibitors; fibrinogen receptor antagonists; prostacyclin mimetics; phosphodiesterase inhibitors; ADP-receptor (P_2T) antagonists; and inhibitors of carboxypeptidase U (CPU).

Compounds of formula (I) that inhibit trypsin and/or thrombin may also be useful in the treatment of pancreatitis.

The formulations of the invention are thus indicated both in the therapeutic and/or prophylactic treatment of these conditions.

According to a further aspect of the present invention, there is provided a method of treatment of a condition where inhibition of thrombin is required which method comprises administration of a therapeutically effective amount of a formulation of the invention to a person suffering from, or susceptible to, such a condition.

In a still further aspect the present invention provides a formulation of the invention in the manufacture of a medicament for use in the treatment of thrombosis.

According to a further aspect of the invention, there is provided a method of treatment of thrombosis which method comprises administration of a formulation of the invention to a person suffering from, or susceptible to, such a condition.

For the avoidance of doubt, by "treatment" we include the therapeutic treatment, as well as the prophylaxis, of a condition.

Suitable amounts of active ingredient in formulations (oral or parenteral), concentrated formulations, and solid compositions, of the invention depend upon many factors, such as the nature of that ingredient (free base/salt etc), the dose that is required in an oral formulation or in a final "ready to use" parenteral formulation that is, or is to be, prepared, and the nature, and amounts, of other constituents of the formulation. However, a typical daily dose of a compound of formula (I), or a pharmaceutically acceptable salt thereof, is in the range 0.001-100 mg/kg body weight at peroral administration and 0.001-50 mg/kg body weight at parenteral administration, excluding the weight of any acid counter-ion, irrespective of the number of individual doses that are administered during the course of that day. In the case of an immediate release parenteral formulation administration may be continuous (for example by way of infusion). A preferred daily oral dose is 20-500mg and a preferred parenteral dose is in the range 0.1-50mg.

General Procedures

TLC was performed on silica gel. Chiral HPLC analysis was performed using a 46 mm X 250 mm Chiralcel OD column with a 5 cm guard column. The column temperature was maintained at 35°C. A flow rate of 1.0 mL/min was used. A Gilson 115 UV detector at 228 nm was used. The mobile phase consisted of hexanes, ethanol and trifluroacetic acid and the appropriate ratios are listed for each compound. Typically, the product was dissolved in a minimal amount of ethanol and this was diluted with the mobile phase.

In Preparations A to I below, LC-MS/MS was performed using a HP-1100 instrument equipped with a CTC-PAL injector and a 5 Tm, 4x100 mm

ThermoQuest, Hypersil BDS-C18 column. An API-3000 (Sciex) MS detector was used. The flow rate was 1.2 mL/min and the mobile phase (gradient) consisted of 10-90% acetonitrile with 90-10% of 4 mM aq. ammonium acetate, both containing 0.2% formic acid. Otherwise, low resolution mass spectra (LRMS) were recorded using a Micromass ZQ spectrometer in ESI posneg switching ion mode (mass range m/z 100-800); and high resolution mass spectra (HRMS) were recorded using a Micromass LCT spectrometer in ES negative ionization mode (mass range m/z 100-1000) with Leucine Enkephalin ($C_{28}H_{37}N_5O_7$) as internal mass standard.

^{1}H NMR spectra were recorded using tetramethylsilane as the internal standard.

Preparation A

Preparation of Compound A

(i) 3-Chloro-5-methoxybenzaldehyde

3,5-Dichloroanisole (74.0 g, 419 mmol) in THF (200 mL) was added dropwise to magnesium metal (14.2 g, 585 mmol, pre-washed with 0.5 N HCl) in THF (100 mL) at 25°C. After the addition, 1,2-dibromoethane (3.9 g, 20.8 mmol) was added dropwise. The resultant dark brown mixture was heated at reflux for 3 h. The mixture was cooled to 0°C, and *N,N*-dimethylformamide (60 mL) was added in one portion. The mixture was partitioned with diethyl ether (3 x 400 mL) and 6N HCl (500 mL). The combined organic extracts were washed with brine (300 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo* to give an oil. Flash chromatography (2x) on silica gel eluting with Hex:EtOAc (4:1) afforded the sub-title compound (38.9 g, 54%) as a yellow oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 9.90 (s, 1H), 7.53 (s, 1H), 7.38 (s, 1H), 7.15 (s, 1H), 3.87 (s, 3H).

(ii) 3-Chloro-5-hydroxybenzaldehyde

A solution of 3-chloro-5-methoxybenzaldehyde (22.8 g, 134 mmol; see step (i) above) in CH_2Cl_2 (250 mL) was cooled to 0°C. Boron tribromide (15.8 mL, 167 mmol) was added dropwise over 15 min. After stirring, the reaction mixture for 2 h, H_2O (50 mL) was added slowly. The solution was then extracted with Et_2O (2 x 100 mL). The organic layers were combined, dried (Na_2SO_4), filtered and concentrated *in vacuo*. Flash chromatography on silica gel eluting with Hex:EtOAc (4:1) afforded the sub-title compound (5.2 g, 25%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 9.85 (s, 1H), 7.35 (s,1H), 7.20 (s,1H), 7.10 (s,1H), 3.68 (s,1H)

(iii) 3-Chloro-5-difluoromethoxybenzaldehyde

A solution of 3-chloro-5-hydroxybenzaldehyde (7.5g, 48 mmol; see step (ii) above) in 2-propanol (250 mL) and 30% KOH (100 mL) was heated to reflux. While stirring, $CHClF_2$ was bubbled into the reaction mixture for 2 h. The reaction mixture was cooled, acidified with 1N HCl and extracted with EtOAc (2 x 100 mL). The organics were washed with brine (100 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo*. Flash chromatography on silica gel eluting with Hex:EtOAc (4:1) afforded the sub-title compound (4.6 g, 46%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 9.95 (s, 1H), 7.72 (s, 1H), 7.52 (s, 1H), 7.40 (s, 1H), 6.60 (t, $J_{H\text{-}F}$ = 71.1 Hz, 1H)

(iv) Ph(3-Cl)(5-$OCHF_2$)-(*R,S*)CH(OTMS)CN

A solution of 3-chloro-5-difluoromethoxybenzaldehyde (4.6 g, 22.3 mmol; see step (iii) above) in CH_2Cl_2 (200 mL) was cooled to 0°C. ZnI_2 (1.8 g, 5.6 mmol) and trimethylsilyl cyanide (2.8 g, 27.9 mmol) were added and the reaction mixture was allowed to warm to room temperature and stirred for 15 h. The mixture was partially concentrated *in vacuo* yielding the sub-title compound as a liquid, which was used directly in step (v) below without further purification or characterization.

(v) Ph(3-Cl)(5-$OCHF_2$)-(*R,S*)CH(OH)C(NH)OEt

Ph(3-Cl)(5-$OCHF_2$)-(*R,S*)CH(OTMS)CN (6.82 g, assume 22.3 mmol; see step (iv) above) was added dropwise to HCl/EtOH (500 mL). The reaction mixture was stirred 15 h, then partially concentrated *in vacuo* yielding the sub-title compound as a liquid, which was used in step (vi) without further purification or characterization.

(vi) Ph(3-Cl)(5-$OCHF_2$)-(*R,S*)CH(OH)C(O)OEt

Ph(3-Cl)(5-$OCHF_2$)-(*R,S*)CH(OH)C(NH)OEt (6.24 g, assume 22.3 mmol; see step (v) above) was dissolved in THF (250 mL), 0.5M H_2SO_4 (400 mL) was added and the reaction was stirred at 40°C for 65 h, cooled and then partially concentrated *in vacuo* to remove most of the THF. The reaction mixture was then extracted with Et_2O (3 x 100 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo* to afford the sub-title compound as a solid, which was used in step (vii) without further purification or characterization.

(vii) Ph(3-Cl)(5-$OCHF_2$)-(*R,S*)CH(OH)C(O)OH

A solution of Ph(3-Cl)(5-$OCHF_2$)-(*R,S*)CH(OH)C(O)OEt (6.25 g, assume 22.3 mmol; see step (vi) above) in 2-propanol (175 mL) and 20% KOH (350 mL) was stirred at room temperature 15 h. The reaction was then partially concentrated *in vacuo* to remove most of the 2-propanol. The remaining mixture was acidified with 1M H_2SO_4, extracted with Et_2O (3 x 100 mL), dried (Na_2SO_4) and concentrated *in vacuo* to give a solid. Flash chromatography on silica gel eluting with $CHCl_3$:MeOH:concentrated NH_4OH (6:3:1) afforded the ammonium salt of the sub-title compound. The ammonium salt was then dissolved in a mixture of EtOAc (75 mL) and H_2O (75 mL) and acidified with 2N HCl. The organic layer was separated and washed with brine (50 mL), dried (Na_2SO_4) and concentrated *in vacuo* to afford the sub-title compound (3.2 g, 57% from steps (iv) to (vii)).

^{1}H NMR (300 MHz, CD_3OD) δ 7.38 (s, 1H), 7.22 (s, 1H), 7.15 (s, 1H), 6.89 (t, J_{H-F} = 71.1 Hz, 1H), 5.16 (s, 1H)

(viii) Ph(3-Cl)(5-$OCHF_2$)-(*R*)CH(OH)C(O)OH (a) and Ph(3-Cl)(5-$OCHF_2$)-(*S*)CH(OAc)C(O)OH (b)

A mixture of Ph(3-Cl)(5-$OCHF_2$)-(*R*,*S*)CH(OH)C(O)OH (3.2 g, 12.7 mmol; see step (vii) above) and Lipase PS "Amano" (~2.0 g) in vinyl acetate (125 mL) and MTBE (125 mL) was heated at reflux for 48 h. The reaction mixture was cooled, filtered through Celite® and the filter cake washed with EtOAc. The filtrate was concentrated *in vacuo* and subjected to flash chromatography on silica gel eluting with $CHCl_3$:MeOH:concentrated NH_4OH (6:3:1) yielding the ammonium salts of the sub-title compounds (a) and (b). Compound (a) as a salt was dissolved in H_2O, acidified with 2N HCl and extracted with EtOAc. The organic layer was washed with brine, dried (Na_2SO_4), filtered and concentrated *in vacuo* to afford the sub-title compound (a) (1.2 g, 37%).

For sub-title compound (a)

^{1}H NMR (300 MHz, CD_3OD) δ 7.38 (s, 1H), 7.22 (s, 1H), 7.15 (s, 1H), 6.89 (t, J_{H-F} = 71.1 Hz, 1H), 5.17 (s, 1H)

(ix) Ph(3-Cl)(5-$OCHF_2$)-(*R*)CH(OH)C(O)-Aze-Pab(Teoc)

To a solution of Ph(3-Cl)(5-$OCHF_2$)-(*R*)CH(OH)C(O)OH (1.1 g, 4.4 mmol; see step (viii) above) and H-Aze-Pab(Teoc) (see international patent application WO 00/42059, 2.6 g, 5.7 mmol) in DMF (50 mL) at 0°C was added PyBOP (2.8 g, 5.3 mmol) and collidine (1.3 g, 10.6 mmol). The reaction was stirred at 0°C for 2 h and then at room temperature for an additional 15 h. The reaction mixture was concentrated *in vacuo* and flash chromatographed on silica gel (3 x), eluting first with $CHCl_3$:EtOH (9:1), then with EtOAc:EtOH (20:1) and finally eluting with CH_2Cl_2:CH_3OH (95:5) to afford the sub-title compound (1.0 g, 37%) as a white solid.

^{1}H NMR (300 MHz, CD_3OD, mixture of rotamers) δ 7.79-7.85 (d, J = 8.7 Hz, 2H), 7.15-7.48 (m, 5H), 6.89 and 6.91 (t, J_{H-F} = 71.1 Hz, 1H), 5.12 and 5.20 (s, 1H), 4.75-4.85 (m, 1H), 3.97-4.55 (m, 6H), 2.10-2.75 (m, 2H), 1.05-1.15 (m, 2H), 0.09 (s, 9H)

MS (m/z) 611 $(M + 1)^+$

(x) Ph(3-Cl)(5-$OCHF_2$)-(*R*)CH(OH)C(O)-Aze-Pab(OMe, Teoc)

Ph(3-Cl)(5-$OCHF_2$)-(*R*)CH(OH)C(O)-Aze-Pab(Teoc) (0.40 g, 0.65 mmol; see step (ix) above), was dissolved in 20 mL of acetonitrile and 0.50 g (6.0 mmol) of O-methyl hydroxylamine hydrochloride was added. The mixture was heated at 70°C for 2 h. The solvent was evaporated and the residue was partitioned between water and ethyl acetate. The aqueous phase was extracted twice more with ethyl acetate and the combined organic phase was washed with water, brine, dried (Na_2SO_4), filtered and evaporated. Yield: 0.41 g (91%).

^{1}H-NMR (400 MHz; $CDCl_3$) : δ 7.83 (bt, 1H), 7.57 (bs, 1H), 7.47 (d, 2H), 7.30 (d, 2H), 7.20 (m, 1H), 7.14 (m, 1H), 7.01 (m, 1H), 6.53 (t, 1H), 4.89 (s, 1H), 4.87 (m, 1H), 4.47 (m, 2H), 4.4-4.2 (b, 1H), 4.17-4.1 (m, 3H), 3.95 (s, 3H), 3.67 (m, 1H), 2.68 (m, 1H), 2.42 (m,1H) 0.97 (m, 2H), 0.01 (s, 9H).

(xi) Compound A

Ph(3-Cl)(5-$OCHF_2$)-(*R*)CH(OH)C(O)-Aze-Pab(OMe, Teoc) (0.40 g, 0.62 mmol; see step (x) above), was dissolved in 5 mL of TFA and allowed to react for 30 min. TFA was evaporated and the residue was partitioned between ethyl acetate and $NaHCO_3$ (aq.). The aqueous phase was extracted twice more with ethyl acetate and the combined organic phase was washed with water, brine, dried (Na_2SO_4), filtered and evaporated. The product was freeze dried from water/acetonitrile. No purification was necessary. Yield: 0.28 g (85%).

^{1}H-NMR (600 MHz; $CDCl_3$) : δ 7.89 (bt, 1H), 7.57 (d, 2H), 7.28 (d, 2H), 7.18 (m, 1H), 7.13 (m,1H), 6.99 (m, 1H), 6.51 (t, 1H), 4.88 (s, 1H), 4.87 (m, 1H), 4.80 (bs, 2H), 4.48 (dd, 1H), 4.43 (dd, 1H), 4.10 (m, 1H), 3.89 (s, 3H), 3.68 (m, 1H), 2.68 (m, 1H), 2.40 (m, 1H).

^{13}C-NMR (125 MHz; $CDCl_3$): (carbonyl and/or amidine carbons, rotamers) δ 172.9, 170.8, 152.7, 152.6

HRMS calculated for $C_{22}H_{23}ClF_2N_4O_5$ $(M-H)^-$ 495.1242, found 495.1247

Preparation B

Preparation of Compound B

(i) 2,6-Difluoro-4[(methylsulfinyl)(methylthio)methyl]benzonitrile

(Methylsulfinyl)(methylthio)methane (7.26g, 0.0584 mol) was dissolved in 100 mL of dry THF under argon and was cooled to –78°C. Butyllithium in hexane (16 mL 1.6M, 0.0256 mol) was added dropwise with stirring. The mixture was stirred for 15 min. Meanwhile, a solution of 3,4,5-trifluorobenzonitrile (4.0 g, 0.025 mmol) in 100 mL of dry THF was cooled to -78°C under argon and the former solution was added through a cannula to the latter solution over a period of 35 min. After 30 min, the cooling bath was removed and when the reaction had reached room temperature it was poured into 400 mL of water. The THF was evaporated and the remaining aqueous layer was extracted three times with diethyl ether. The combined ether phase was washed with water, dried (Na_2SO_4) and evaporated. Yield: 2.0 g (30%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.4-7.25 (m, 2H), 5.01 (s, 1H, diasteromer), 4.91 (s, 1H, diasteromer), 2.88 (s, 3H, diasteromer), 2.52 (s, 3H, diasteromer), 2.49 (s, 3H, diasteromer), 2.34 (s, 3H, diasteromer), 1.72 (broad, 1H)

(ii) 2,6-Difluoro-4-formylbenzonitrile

2,6-Difluoro-4[(methylsulfinyl)(methylthio)methyl]benzonitrile (2.17 g, 8.32 mmol; see step (i) above) was dissolved in 90 mL of THF and 3.5 mL of concentrated sulfuric acid was added. The mixture was left at room temperature for 3 days and subsequently poured into 450 mL of water. Extraction three times with EtOAc followed and the combined ethereal phase was washed twice with aqueous sodium bicarbonate and with brine, dried (Na_2SO_4) and evaporated. Yield: 1.36 g (98%). The position of the formyl group was established by ^{13}C NMR. The signal from the fluorinated carbons at 162.7 ppm exhibited the expected coupling pattern with two coupling constants in the order of 260 Hz and 6.3 Hz respectively corresponding to an *ipso* and a *meta* coupling from the fluorine atoms.

^1H NMR (400 MHz, $CDCl_3$) δ 10.35 (s, 1H), 7.33 (m, 2H)

(iii) 2,6-Difluoro-4-hydroxymethylbenzonitrile

2,6-Difluoro-4-formylbenzonitrile (1.36 g, 8.13 mmol; see step (ii) above) was dissolved in 25 mL of methanol and cooled on an ice bath. Sodium borohydride (0.307 g, 8.12 mmol) was added in portions with stirring and the reaction was left for 65 min. The solvent was evaporated and the residue was partitioned between diethyl ether and aqueous sodium bicarbonate. The ethereal layer was washed with more aqueous sodium bicarbonate and brine, dried (Na_2SO_4) and evaporated. The crude product crystallised soon and could be used without further purification. Yield: 1.24 g (90%).

^1H NMR (400 MHz, $CDCl_3$) δ 7.24 (m, 2H), 4.81 (s, 2H), 2.10 (broad, 1H)

(iv) 4-Cyano-2,6-difluorobenzyl methanesulfonate

To an ice cooled solution of 2,6-difluoro-4-hydroxymethylbenzonitrile (1.24 g, 7.32 mmol; see step (iii) above) and methanesulfonyl chloride (0.93 g, 8.1 mmol) in 60 mL of methylene chloride was added triethylamine (0.81 g, 8.1 mmol) with

stirring. After 3 h at 0°C, the mixture was washed twice with 1M HCl and once with water, dried (Na_2SO_4) and evaporated. The product could be used without further purification. Yield: 1.61 g (89%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.29 (m, 2H), 5.33 (s, 2H), 3.07 (s, 3H)

(v) 4-Azidomethyl-2,6-difluorobenzonitrile

A mixture of 4-cyano-2,6-difluorobenzyl methanesulfonate (1.61 g, 6.51 mmol; see step (iv) above) and sodium azide (0.72 g, 0.0111 mol) in 10 mL of water and 20 mL of DMF was stirred at room temperature overnight. The resultant was subsequently poured into 200 mL of water and extracted three times with diethyl ether. The combined ethereal phase was washed five times with water, dried (Na_2SO_4) and evaporated. A small sample was evaporated for NMR purposes and the product crystallised. The rest was evaporated cautiously but not until complete dryness. Yield (theoretically 1.26 g) was assumed to be almost quantitative based on NMR and analytical HPLC.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29 (m, 2H), 4.46 (s, 2H)

(vi) 4-Aminomethyl-2,6-difluorobenzonitrile

This reaction was carried out according to the procedure described in *J. Chem. Res. (M)* (1992) 3128. To a suspension of 520 mg of 10% Pd/C (50% moisture) in 20 mL of water was added a solution of sodium borohydride (0.834 g, 0.0221 mol) in 20 mL of water. Some gas evolution resulted. 4-Azidomethyl-2,6-difluorobenzonitrile (1.26 g, 6.49 mmol; see step (v) above) was dissolved in 50 mL of THF and added to the aqueous mixture on an ice bath over 15 min. The mixture was stirred for 4 h, whereafter 20 mL of 2M HCl was added and the mixture was filtered through Celite. The Celite was rinsed with more water and the combined aqueous phase was washed with EtOAc and subsequently made alkaline with 2M NaOH. Extraction three times with methylene chloride followed

and the combined organic phase was washed with water, dried (Na_2SO_4) and evaporated. Yield: 0.87 g (80%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.20 (m, 2H), 3.96 (s, 2H), 1.51 (broad, 2H)

(vii) 2,6-Difluoro-4-*tert*-butoxycarbonylaminomethylbenzonitrile

A solution of 4-aminomethyl-2,6-difluorobenzonitrile (0.876 g, 5.21 mmol; see step (vi) above) was dissolved in 50 mL of THF and di-*tert*-butyl dicarbonate (1.14 g , 5.22 mmol) in 10 mL of THF was added. The mixture was stirred for 3.5 h. The THF was evaporated and the residue was partitioned between water and EtOAc. The organic layer was washed three times with 0.5 M HCl and water, dried (Na_2SO_4) and evaporated. The product could be used without further purification. Yield: 1.38 g (99%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.21 (m,2H), 4.95 (broad, 1H), 4.43 (broad, 2H), 1.52 (s, 9H)

(viii) Boc-Pab(2,6-diF)(OH)

A mixture of 2,6-difluoro-4-*tert*-butoxycarbonylaminomethylbenzonitrile (1.38 g, 5.16 mmol; see step (vii) above), hydroxylamine hydrochloride (1.08 g, 0.0155 mol) and triethylamine (1.57 g, 0.0155 mol) in 20 mL of ethanol was stirred at room temperature for 36 h. The solvent was evaporated and the residue was partitioned between water and methylene chloride. The organic layer was washed with water, dried (Na_2SO_4) and evaporated. The product could be used without further purification. Yield: 1.43 g (92%).

^{1}H NMR (500 MHz, CD_3OD) δ 7.14 (m, 2H), 4.97 (broad, 1H), 4.84 (broad, 2H), 4.40 (broad, 2H), 1.43 (s, 9H)

(ix) Boc-Pab(2,6-diF) x HOAc

This reaction was carried out according to the procedure described by Judkins *et al, Synth. Comm.* (1998) 4351. Boc-Pab(2,6-diF)(OH) (1.32 g, 4.37 mmol; see step (viii) above), acetic anhydride (0.477 g, 4.68 mmol) and 442 mg of 10% Pd/C (50% moisture) in 100 mL of acetic acid was hydrogenated at 5 atm pressure for 3.5 h. The mixture was filtered through Celite, rinsed with ethanol and evaporated. The residue was freeze-dried from acetonitrile and water and a few drops of ethanol. The sub-title product could be used without further purification. Yield: 1.49 g (99%).

^{1}H NMR (400 MHz, CD_3OD) δ 7.45 (m, 2H), 4.34 (s, 2H), 1.90 (s, 3H), 1.40 (s, 9H)

(x) Boc-Pab(2,6-diF)(Teoc)

To a solution of Boc-Pab(2,6-diF) x HOAc (1.56 g, 5.49 mmol; see step (ix) above) in 100 mL of THF and 1 mL of water was added 2-(trimethylsilyl)ethyl p-nitrophenyl carbonate (1.67 g, 5.89 mmol). A solution of potassium carbonate (1.57 g, 0.0114 mol) in 20 mL of water was added dropwise over 5 min. The mixture was stirred overnight. The THF was evaporated and the residue was partitioned between water and methylene chloride. The aqueous layer was extracted with methylene chloride and the combined organic phase was washed twice with aqueous sodium bicarbonate, dried (Na_2SO_4) and evaporated. Flash chromatography on silica gel with heptane/EtOAc = 2/1 gave 1.71 g (73%) of pure compound.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 (m, 2H), 4.97 (broad, 1H), 4.41 (broad, 2H), 4.24 (m, 2H), 1.41 (s, 9H), 1.11 (m, 2H), 0.06 (s, 9H)

(xi) Boc-Aze-Pab(2,6-diF)(Teoc)

Boc-Pab(2,6-diF)(Teoc) (1.009 g, 2.35 mmol; see step (x) above) was dissolved in 50 mL of EtOAc saturated with HCl(g). The mixture was left for 10 min.,

evaporated and dissolved in 18 mL of DMF, and then cooled on an ice bath. Boc-Aze-OH (0.450 g, 2.24 mmol), PyBOP (1.24 g, 2.35 mmol) and lastly diisopropylethyl amine (1.158 g, 8.96 mmol) were added. The reaction mixture was stirred for 2 h and then poured into 350 mL of water and extracted three times with EtOAc. The combined organic phase was washed with brine, dried (Na_2SO_4) and evaporated. Flash chromatography on silica gel with heptane:EtOAc (1:3) gave 1.097 g (96%) of the desired compound.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.46 (m, 2H), 4.65-4.5 (m, 3H), 4.23 (m, 2H), 3.87 (m, 1H), 3.74 (m, 1H), 2.45-2.3 (m, 2H), 1.40 (s, 9H), 1.10 (m, 2H), 0.05 (s, 9H)

(xii) Ph(3-Cl)(5-$OCHF_2$)-*(R)*CH(OH)C(O)-Aze-Pab(2,6-diF)(Teoc)

Boc-Aze-Pab(2,6-diF)(Teoc) (0.256 g, 0.500 mmol; see step (xi) above) was dissolved in 20 mL of EtOAc saturated with HCl(g). The mixture was left for 10 min. and evaporated and dissolved in 5 mL of DMF. Ph(3-Cl)(5-$OCHF_2$)-*(R)*CH(OH)C(O)OH (0.120 g, 0.475 mmol; see Preparation A(viii) above), PyBOP (0.263 g, 0.498 mmol) and lastly diisopropylethyl amine (0.245 g, 1.89 mmol) were added. The reaction mixture was stirred for 2 h and then poured into 350 mL of water and extracted three times with EtOAc. The combined organic phase was washed with brine, dried (Na_2SO_4) and evaporated. Flash chromatography on silica gel with EtOAc gave 0.184 g (60%) of the desired sub-title compound.

^{1}H NMR (400 MHz, CD_3OD, mixture of rotamers) δ 7.55-7.45 (m, 2H), 7.32 (m, 1H, major rotamer), 7.27 (m, 1H, minor rotamer), 7.2-7.1 (m, 2H), 6.90 (t, 1H, major rotamer), 6.86 (t, 1H, minor rotamer), 5.15 (s, 1H,major rotamer), 5.12 (m, 1H, minor rotamer), 5.06 (s, 1H, minor rotamer), 4.72 (m, 1H, major rotamer), 4.6-4.45 (m, 2H), 4.30 (m, 1H, major rotamer), 4.24 (m, 2H), 4.13 (m, 1H, major rotamer), 4.04 (m, 1H, minor rotamer), 3.95 (m, 1H, minor rotamer), 2.62 (m, 1H,

minor rotamer), 2.48 (m, 1H, major rotamer), 2.22 (m, 1H, major rotamer), 2.10 (m, 1H, minor rotamer), 1.07 (m, 2H), 0.07 (m, 9H)

(xiii) Ph(3-Cl)(5-$OCHF_2$)-*(R)*CH(OH)C(O)-Aze-Pab(2,6-diF)(OMe,Teoc)

A mixture of Ph(3-Cl)(5-$OCHF_2$)-*(R)*CH(OH)C(O)-Aze-Pab(2,6-diF)(Teoc) (64 mg, 0.099 mmol; see step (xii) above) and O-methyl hydroxylamine hydrochloride (50 mg, 0.60 mmol) in 4 mL of acetonitrile was heated at 70°C for 3 h. The solvent was evaporated and the residue was partitioned between water and EtOAc. The aqueous layer was extracted twice with EtOAc and the combined organic phase was washed with water, dried (Na_2SO_4) and evaporated. The product could be used without further purification. Yield: 58 mg (87%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (bt, 1H), 7.46 (m, 1H), 7.25-6.95 (m, 5H), 6.51, t, 1H), 4.88 (s, 1H), 4.83 (m, 1H), 4.6-4.5 (m, 2H), 4.4-3.9 (m, 4H), 3.95 (s, 3H), 3.63 (m, 1H), 2.67 (m, 1H), 2.38 (m, 1H), 1.87 (broad, 1H), 0.98 (m, 2H), 0.01, s, 9H)

(xiv) Compound B

Ph(3-Cl)(5-$OCHF_2$)-*(R)*CH(OH)C(O)-Aze-Pab(2,6-diF)(OMe,Teoc) (58 mg, 0.086 mmol; see step (xiii) above) was dissolved in 3 mL of TFA, cooled on an ice bath and allowed to react for 2 h. The TFA was evaporated and the residue dissolved in EtOAc. The organic layer was washed twice with aqueous sodium carbonate and water, dried (Na_2SO_4) and evaporated. The residue was freeze-dried from water and acetonitrile to give 42 mg (92%) of the title compound.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.95 (bt, 1H), 7.2-7.1 (m, 4H), 6.99 (m, 1H), 6.52 (t, 1H), 4.88 (s, 1H), 4.85-4.75 (m, 3H), 4.6-4.45 (m, 2H), 4.29 (broad, 1H), 4.09 (m, 1H), 3.89 (s, 3H), 3.69 (m, 1H), 2.64 (m, 1H), 2.38 (m, 1H), 1.85 (broad, 1H)
^{13}C-NMR (100 MHz; $CDCl_3$): (carbonyl and/or amidine carbons) δ 172.1, 169.8, 151.9

APCI-MS: (M + 1) = 533/535 m/z

Preparation C

Preparation of Compound C

(i) (2-Monofluoroethyl) methanesulfonate

To a magnetically stirred solution of 2-fluoroethanol (5.0 g, 78.0 mmol) in CH_2Cl_2 (90 mL) under nitrogen at 0°C was added triethylamine (23.7 g, 234 mmol) and methanesulfonyl chloride (10.7 g, 93.7 mmol). The mixture was stirred at 0°C for 1.5 h, diluted with CH_2Cl_2 (100 mL) and washed with 2N HCl (100 mL). The aqueous layer was extracted with CH_2Cl_2 (50 mL) and the combined organic extracts washed with brine (75 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo* to afford the sub-title compound (9.7 g, 88%) as a yellow oil which was used without further purification.

^1H NMR (300 MHz, $CDCl_3$) δ 4.76 (t, J = 4 Hz, 1H), 4.64 (t, J = 4 Hz, 1H), 4.52 (t, J = 4 Hz, 1H), 4.43 (t, J = 4 Hz, 1H), 3.09 (s, 3H).

(ii) 3-Chloro-5-monofluoroethoxybenzaldehyde

To a solution of 3-chloro-5-hydroxybenzaldehyde (8.2 g, 52.5 mmol; see Preparation A(ii) above) and potassium carbonate (9.4 g, 68.2 mmol) in DMF (10 mL) under nitrogen was added a solution of (2-monofluoroethyl) methanesulfonate (9.7 g, 68.2 mmol; see step (i) above) in DMF (120 mL) dropwise at room temperature. The mixture was heated to 100°C for 5 h and then stirred overnight at room temperature. The reaction was cooled to 0°C, poured into ice-cold 2N HCl and extracted with EtOAc. The combined organic extracts were washed with brine, dried (Na_2SO_4), filtered and concentrated *in vacuo*. The brown oil was chromatographed on silica gel eluting with Hex:EtOAc (4:1) to afford the sub-title compound (7.6 g, 71%) as a yellow oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 9.92 (s, 1H), 7.48 (s, 1H), 7.32 (s, 1H), 7.21 (s, 1H), 4.87 (t, J = 4 Hz, 1H), 4.71 (t, J = 3 Hz, 1H), 4.33 (t, J = 3 Hz, 1H), 4.24 (t, J = 3 Hz, 1H).

(iii) Ph(3-Cl)(5-OCH_2CH_2F)-(*R*,*S*)CH(OTMS)CN

To a solution of 3-chloro-5-monofluoroethoxybenzaldehyde (7.6 g, 37.5 mmol; see step (ii) above) and zinc iodide (3.0 g, 9.38 mmol) in CH_2Cl_2 (310 mL) was added trimethylsilyl cyanide (7.4 g, 75.0 mmol) dropwise at 0°C under nitrogen. The mixture was stirred at 0°C for 3 h and at room temperature overnight. The reaction was diluted with H_2O (300 mL), the organic layer was separated, dried (Na_2SO_4), filtered and concentrated *in vacuo* to afford the sub-title compound (10.6 g, 94%) as a brown oil that was used without further purification or characterisation.

(iv) Ph(3-Cl)(5-OCH_2CH_2F)-(*R*,*S*)CH(OH)C(O)OH

Concentrated hydrochloric acid (100 mL) was added to Ph(3-Cl)(5-OCH_2CH_2F)-(*R*,*S*)CH(OTMS)CN (10.6 g, 5.8 mmol; see step (iii) above) and the solution stirred at 100°C for 3 h. After cooling to room temperature, the reaction was further cooled to 0°C, basified slowly with 3N NaOH (~300 mL) and washed with Et_2O (3 x 200 mL). The aqueous layer was acidified with 2N HCl (80 mL) and extracted with EtOAc (3 x 300 mL). The combined EtOAc extracts were dried (Na_2SO_4), filtered and concentrated *in vacuo* to afford the sub-title compound (8.6 g, 98%) as a pale yellow solid that was used without further purification.

R_f = 0.28 (90:8:2 $CHCl_3$:MeOH:concentrated NH_4OH)

^{1}H NMR (300 MHz, CD_3OD) δ 7.09 (s, 1H), 7.02 (s, 1H), 6.93 (s, 1H), 5.11 (s, 1H), 4.77-4.81 (m, 1H), 4.62-4.65 (m, 1H), 4.25-4.28 (m, 1H), 4.15-4.18 (m, 1H).

(v) Ph(3-Cl)(5-OCH_2CH_2F)-(*S*)CH(OAc)C(O)OH (a) and Ph(3-Cl)(5-OCH_2CH_2F)-(*R*)CH(OH)C(O)OH (b)

A solution of Ph(3-Cl)(5-OCH_2CH_2F)-(*R*,*S*)CH(OH)C(O)OH (8.6 g, 34.5 mmol; see step (iv) above) and Lipase PS "Amano" (4.0 g) in vinyl acetate (250 mL) and MTBE (250 mL) was heated at 70°C under nitrogen for 3 d. The reaction was cooled to room temperature and the enzyme removed by filtration through Celite®. The filter cake was washed with EtOAc and the filtrate concentrated *in vacuo*. Chromatography on silica gel eluting with $CHCl_3$:MeOH:Et_3N (90:8:2) afforded the triethylamine salt of sub-title compound (a) as a yellow oil. In addition, the triethylamine salt of sub-title compound (b) (4.0 g) was obtained. The salt of sub-title compound (b) was dissolved in H_2O (250 mL), acidified with 2N HCl and extracted with EtOAc (3 x 200 mL). The combined organic extracts were dried (Na_2SO_4), filtered and concentrated *in vacuo* to yield the sub-title compound (b) (2.8 g, 32%) as a yellow oil.

Data for Sub-Title Compound (b):

R_f = 0.28 (90:8:2 $CHCl_3$:MeOH:concentrated NH_4OH)

^1H NMR (300 MHz, CD_3OD) δ 7.09 (s, 1H), 7.02 (s, 1H), 6.93 (s, 1H), 5.11 (s, 1H), 4.77-4.81 (m, 1H), 4.62-4.65 (m, 1H), 4.25-4.28 (m, 1H), 4.15-4.18 (m, 1H).

(vi) Compound C

To a solution of Ph(3-Cl)(5-OCH_2CH_2F)-(*R*)CH(OH)C(O)OH (818 mg, 3.29 mmol; see step (v) above) in DMF (30 mL) under nitrogen at 0°C was added HAze-Pab(OMe)•2HCl (1.43 g, 4.27 mmol, see international patent application WO 00/42059), PyBOP (1.89 g, 3.68 mmol), and DIPEA (1.06 g, 8.23 mmol). The reaction was stirred at 0°C for 2 h and then at room temperature overnight. The mixture was concentrated *in vacuo* and the residue chromatographed two times on silica gel, eluting first with $CHCl_3$:EtOH (15:1) and second with EtOAc:EtOH (20:1) to afford the title compound (880 mg, 54%).

R_f = 0.60 (10:1 $CHCl_3$:EtOH)

^{1}H NMR (300 MHz, CD_3OD, complex mixture of rotamers) δ 7.58-7.60 (d, J = 8 Hz, 2H), 7.34 (d, J = 7 Hz, 2H), 7.05-7.08 (m, 2H), 6.95-6.99 (m, 1H), 5.08-5.13 (m, 1H), 4.77-4.82 (m, 1H), 4.60-4.68 (m, 1H), 3.99-4.51 (m, 7H), 3.82 (s, 3H), 2.10-2.75 (m, 2H).

^{13}C-NMR (150 MHz; CD_3OD): (carbonyl and/or amidine carbons) δ 173.3, 170.8, 152.5.

APCI-MS: (M + 1) = 493 m/z.

Preparation of Compound D (Ph(3-Cl)(5-$OCHF_2$)-(*R*)CH(OH)C(O)-Aze-Pab)

Compound D

Ph(3-Cl)(5-$OCHF_2$)-(*R*)CH(OH)C(O)-Aze-Pab(Teoc) (0.045 g, 0.074 mmol; see Preparation A (ix) above), was dissolved in 3 mL of TFA and allowed to react for 1 h. TFA was evaporated and the residue was freeze dried from water/acetonitrile to yield 0.043 g (100%) of the sub-title compound as its TFA salt.

^{1}H-NMR (400 MHz; CD_3OD) rotamers: δ 7.8-7.75 (m, 2H), 7.55-7.5 (m, 2H), 7.35 (m, 1H, major rotamer), 7.31 (m, 1H, minor rotamer), 7.19 (m, 1H, major rotamer), 7.15 (m, 1H), 7.12 (m, 1H, minor rotamer), 6.89 (t, 1H, major rotamer), 6.87 (t, 1H, minor rotamer), 5.22 (m, 1H, minor rotamer), 5.20 (s, 1H, major rotamer), 5.13 (s, 1H, minor rotamer), 4.80 (m, 1H, major rotamer), 4.6-4.4 (m, 2H), 4.37 (m, 1H, major rotamer), 4.19 (m, 1H, major rotamer), 4.07 (m, 1H, minor rotamer), 3.98 (m, 1H, minor rotamer), 2.70 (m, 1H, minor rotamer), 2.55 (m, 1H, major rotamer), 2.29 (m, 1H, major rotamer), 2.15 (m, 1H, minor rotamer)

^{13}C-NMR (100 MHz; CD_3OD): (carbonyl and/or amidine carbons, rotamers) δ 172.6, 172.5, 172.0, 171.7, 167.0

MS (m/z) 465 $(M-1)^-$, 467 $(M+1)^+$

Preparation of Compound E (Ph(3-Cl)(5-$OCHF_2$)-(*R*)CH(OH)C(O)-Aze-Pab(2,6-diF))

Compound E

Ph(3-Cl)(5-$OCHF_2$)-(*R*)CH(OH)C(O)-Aze-Pab(2,6-diF)(Teoc) (81 mg, 0.127 mmol; see Preparation B (xii) above) was dissolved in 0.5 mL of methylene chloride and cooled on an ice bath. TFA (3 mL) was added and the reaction was left for 75 min. The TFA was evaporated and the residue was freeze dried from water and acetonitrile. The crude product was purified by preparative RPLC with CH_3CN:0.1M NH_4OAc (35:65) to produce 39 mg (55%) of the title compound as its HOAc salt, purity: 99%.

^1H NMR (400 MHz, CD_3OD mixture of rotamers) δ 7.5-7.4 (m, 2H), 7.32 (m, 1H, major rotamer), 7.28 (m, 1H, minor rotamer), 7.2-7.1 (m, 3H) 6.90 (t, 1H, major rotamer), 6.86 (t, minor rotamer), 5.15 (s, 1H, major rotamer), 5.14 (m, 1H, minor rotamer), 5.07 (s, 1H, minor rotamer), 4.72 (m, 1H, major rotamer), 4.65-4.45 (m, 2H), 4.30 (m, 1H, major rotamer), 4.16 (m, 1H, major rotamer), 4.03 (m, 1H, minor rotamer), 3.95 (m, 1H, minor rotamer), 2.63 (m, 1H, minor rotamer), 2.48 (m, 1H, major rotamer), 2.21 (m, 1H, major rotamer), 2.07 (m, 1H, minor rotamer), 1.89 (s, 3H)

^{13}C-NMR (75 MHz; CD_3OD): (carbonyl and/or amidine carbons, mixture of rotamers) δ 171.9, 171.2, 165.0, 162.8, 160.4

APCI-MS: (M + 1) = 503/505 m/z.

Preparation of Compound F (Ph(3-Cl)(5-OCH_2CH_2F)-(*R*)CH(OH)C(O)-Aze-Pab x TFA)

(i) Ph(3-Cl)(5-OCH_2CH_2F)-(*R*)CH(OH)C(O)-Aze-Pab(Teoc)

To a solution of Ph(3-Cl)(5-OCH_2CH_2F)-(*R*)CH(OH)C(O)OH (940 mg, 3.78 mmol; see Preparation C (v) above) in DMF (30 mL) under nitrogen at 0°C was added HAze-Pab(Teoc)•HCl (2.21 g, 4.91 mmol), PyBOP (2.16 g, 4.15 mmol), and DIPEA (1.22 g, 9.45 mmol). The reaction was stirred at 0°C for 2 h and then

at room temperature for 4 h. The mixture was concentrated *in vacuo* and the residue chromatographed twice on silica gel, eluting first with $CHCl_3$:EtOH (15:1) and second with EtOAc:EtOH (20:1) to afford the sub-title compound (450 mg, 20%) as a crushable white foam.

Mp: 80-88°C

R_f = 0.60 (10:1 $CHCl_3$:EtOH)

^{1}H NMR (300 MHz, CD_3OD, complex mixture of rotamers) δ 7.79 (d, J = 8 Hz, 2H), 7.42 (d, J = 8 Hz, 2H), 7.05-7.08 (m, 1H), 6.93-6.99 (m, 2H), 5.08-5.13 (m, 1H), 4.75-4.80 (m, 2H), 4.60-4.68 (m, 1H), 3.95-4.55 (m, 8H), 2.10-2.75 (m, 2H), 1.05-1.11 (m, 2H), 0.08 (s, 9H).

APCI-MS: (M + 1) = 607 m/z.

(ii) Compound F

Ph(3-Cl)(5-OCH_2CH_2F)-(*R*)CH(OH)C(O)-Aze-Pab(Teoc) (0.357 g, 0.589 mmol; see step (i) above), was dissolved in 10 mL of TFA and allowed to react for 40 min. TFA was evaporated and the residue was freeze dried from water/acetonitrile to yield 0.33 g (93%) of the title compound as its TFA salt.

^{1}H-NMR (600 MHz; CD_3OD) rotamers: δ 7.8-7.7 (m, 2H), 7.54 (d, 2H), 7.08 (s, 1H, major rotamer), 7.04 (s, 1H, minor rotamer), 6.99 (s, 1H, major rotamer), 6.95 (s, 1H), 6.92 (s, 1H, minor rotamer), 5.18 (m, 1H, minor rotamer), 5.14 (s, 1H, major rotamer), 5.08 (s, 1H, minor rotamer), 4.80 (m, 1H, major rotamer), 4.73 (m, 1H), 4.65 (m, 1H), 4.6-4.4 (m, 2H), 4.35 (m, 1H, major rotamer), 4.21 (doublet of multiplets, 2H), 4.12 (m, 1H, major rotamer), 4.06 (m, 1H, minor rotamer), 3.99 (m, 1H, minor rotamer), 2.69 (m, 1H, minor rotamer), 2.53 (m, 1H, major rotamer), 2.29 (m, 1H, major rotamer), 2.14 (m, 1H, minor rotamer).

^{13}C-NMR (150 MHz; CD_3OD): (carbonyl and/or amidine carbons) δ 172.8, 172.1, 167.4.

ESI-MS+: (M+1) = 463 (m/z)

Preparation of Compound G (Ph(3-Cl)(5-$OCHF_2$)-(*R*)CH(OH)C(O)-Aze-Pab(OH))

(i) Ph(3-Cl)(5-$OCHF_2$)-(*R*)CH(OH)C(O)-Aze-Pab(OH, Teoc)

Ph(3-Cl)(5-$OCHF_2$)-(*R*)CH(OH)C(O)-Aze-Pab(Teoc) (0.148 g, 0.24 mmol; see Preparation A step (ix) above), was dissolved in 9 mL of acetonitrile and 0.101 g (1.45 mmol) of hydroxylamine hydrochloride was added. The mixture was heated at 70°C for 2.5 h, filtered through Celite® and evaporated. The crude product (0.145 g; 75% pure) was used directly in the next step without further purification.

(ii) Ph(3-Cl)(5-$OCHF_2$)-(*R*)CH(OH)C(O)-Aze-Pab(OH)

Ph(3-Cl)(5-$OCHF_2$)-(*R*)CH(OH)C(O)-Aze-Pab(OH, Teoc) (0.145 g, 0.23 mmol; see step (i) above), was dissolved in 0.5 mL of CH_2Cl_2 and 9 mL of TFA. The reaction was allowed to proceed for 60 minutes. TFA was evaporated and the residue was purified using preparative HPLC. The fractions of interest were pooled and freeze-dried (2x), yielding 72 mg (yield over two steps 62%) of the title compound.

MS (m/z) 482 $(M - 1)^-$; 484 $(M + 1)^+$

^{1}H-NMR (400 MHz; CD_3OD): δ 7.58 (d, 2H), 7.33 (m, 3H), 7.15 (m, 2H), 6.89 (t, 1H major rotamer), 6.86 (t, 1H minor rotamer), 5.18 (s, 1H major rotamer; and m, 1H minor rotamer), 5.12 (s, 1H minor rotamer), 4.77 (m, 1H major rotamer),4.42 (m, 2H), 4.34 (m, 1H major rotamer), 4.14 (m, 1H major rotamer), 4.06 (m, 1H minor rotamer), 3.95 (m, 1H minor rotamer), 2.66 (m, 1H minor rotamer), 2.50 (m, 1H major rotamer), 2.27 (m, 1H major rotamer), 2.14 (m, 1H minor rotamer)

^{13}C-NMR (100 MHz; CD_3OD): (carbonyl and/or amidine carbons, rotamers) δ 172.4, 172.3, 172.0, 171.4 152.3, 152.1

Preparation of Compound J (Ph(3-Cl)(5-OCH_2CHF_2)-*(R)*CH(OH)C(O)-Aze-Pab(OH))

(i) Ph(3-Cl)(5-OCH_2CHF_2)-*(R)*CH(OH)C(O)-Aze-Pab(Z)

Boc-Aze-Pab(Z) (see international patent application WO 97/02284, 92 mg, 0.197 mmol) was dissolved in 10 mL of EtOAc saturated with HCl(g) and allowed to react for 10 min. The solvent was evaporated and the residue was mixed with Ph(3-Cl)(5-OCH_2CHF_2)-*(R)*CH(OH)C(O)OH (50 mg, 0.188 mmol; see Preparation C (v) above), PyBOP (109 mg, 0.209 mmol) and finally diisopropylethyl amine (96 mg, 0.75 mmol) in 2 mL of DMF. The mixture was stirred for 2 h and then poured into 50 mL of water and extracted three times with EtOAc. The combined organic phase was washed with water, dried (Na_2SO_4) and evaporated. The crude product was flash chromatographed on silica gel with EtOAc:MeOH (9:1). Yield: 100 mg (87%).

^{1}H NMR (300 MHz, CD_3OD, mixture of rotamers) δ 7.85-7.75 (m, 2H), 7.45-7.25 (m, 7H), 7.11 (m, 1H, major rotamer), 7.08 (m, 1H, minor rotamer), 7.05-6.9 (m, 2H), 6.13 (bt, 1H), 5.25-5.05 (m, 3H), 4.77 (m, 1H, partially hidden by the CD_3OH signal), 4.5-3.9 (m, 7H), 2.64 (m, 1H, minor rotamer), 2.47 (m, 1H, major rotamer), 2.25 (m, 1H, major rotamer), 2.13 (m, 1H, minor rotamer)

(ii) Ph(3-Cl)(5-OCH_2CHF_2)-*(R)*CH(OH)C(O)-Aze-Pab(OH)

Hydroxylamine hydrochloride (65 mg, 0.94 mmol) and triethylamine (0.319 g, 3.16 mmol) were mixed in 8 mL of THF and sonicated for 1 h at 40°C. Ph(3-Cl)(5-OCH_2CHF_2)-*(R)*CH(OH)C(O)-Aze-Pab(Z) (96 mg, 0.156 mmol; see step (i) above) was added with 8 mL more of THF. The mixture was stirred at 40°C for 4.5 days. The solvent was evaporated and the crude product was purified by preparative RPLC with CH_3CN:0.1M NH_4OAc (40:60). Yield: 30 mg (38%). Purity: 99%.

^{1}H NMR (300 MHz, CD_3OD, mixture of rotamers) δ 7.6-7.55 (m, 2H), 7.35-7.3 (m, 2H), 7.12 (m, 1H, major rotamer), 7.09 (m, 1H, minor rotamer), 7.05-6.9 (m, 2H), 6.15 (triplet of multiplets, 1H), 5.15 (m, 1H, minor rotamer), 5.13 (s, 1H, major rotamer), 5.08 (s, 1H, minor rotamer), 4.77 (m, 1H, major rotamer), 4.5-4.2 (m, 5H), 4.08 (m, 1H, major rotamer), 3.97 (m, 1H, minor rotamer), 2.66 (m, 1H, minor rotamer), 2.50 (m, 1H major rotamer), 2.27 (m, 1H, major rotamer), 2.14 (m, 1H, minor rotamer).

^{13}C-NMR (100 MHz; CD_3OD): (carbonyl and/or amidine carbons, mixture of rotamers) δ 172.8, 172.2, 171.4, 159.1, 158.9, 154.2.

APCI-MS: (M + 1) = 497/499 m/z

Methods 1 and 2

Preparation of Salts of Compound A

Method 1

General Method for Salt Preparation

The following generic method was employed to prepare salts of Compound A: 200 mg of Compound A (see Preparation A above) was dissolved in 5 mL of MeOH. To this solution was added a solution of the relevant acid (1.0 molar equivalent) dissolved in 5 mL of MeOH. After stirring for 10 minutes at room temperature, the solvent was removed by way of a rotary evaporator. The remaining solid material was re-dissolved in 8 mL of acetonitrile:H_2O (1:1). Freeze-drying afforded colorless amorphous material in each case.

Acids employed:

(1S)-(+)-10-camphorsulfonic

malic

cyclohexylsulphamic

phosphoric

dimethylphosphoric

p-toluenesulphonic

L-lysine

L-lysine hydrochloride

saccharinic

methanesulphonic

hydrochloric

Appropriate characterising data are shown in Table 1.

Table 1

Salt	Mw acid	Mw salt	LRMS	δ ppm (MeOD) H18, H19, H24 (see structure at end of Method 9 below)
(1*S*)-(+)-10-camphorsulfonic acid	232.20	729.20	230.9 495.1 497.0 727.3	7.57, 7.68, 3.97
maleate	116.07	612.97	114.8 495.1 497.0	7.45, 7.64, 3.89
cyclohexylsulphamate	179.24	676.14	177.9 495.1 496.9 674.3 676.1	7.44, 7.64, 3.89
phosphate	97.99	594.89	495.1 497.0 593.1	7.37, 7.61, 3.84

dimethylphosphate	126.05	622.95	124.9 495.1 497.0 621.2 623.0	7.50, 7.66, 3.92
p-toluenesulphonate	172.20	669.10	170.9 495.1 497.0	7.54, 7.71, 3.95
L-lysine	146.19	643.09	145.0 495.1 497.0	7.36, 7.60, 3.83
L-lysine hydrochloride	182.65	679.55	495.1 497.0 531.1 (HCl)	7.36, 7.60, 3.83
saccharinate	183.19	680.09	181.9 495.1 497.0	7.44, 7.64. 3.89
methanesulphonate	96.11	593.01	495.1 497.0 591.2 593.1	7.57, 7.68, 3.97
hydrochloride	36.46	533.36	495.1 496.9 531.1 532.5 535.2	7.55, 7.67, 3.95

All salts formed in this Method were amorphous.

Method 2

Further amorphous salts of Compound A were made using analogous techniques to those described in Method 1 above from the following acids:

hydrobromic acid (1:1 salt)

hydrochloric acid (1:1 salt)

sulphuric acid (1:0.5 salt)

1,2-ethanedisulfonic acid (1.0.5 salt)

1S-camphorsulfonic acid (1:1 salt)

(+/-)-camphorsulfonic acid (1:1 salt)

ethanesulfonic acid (1:1 salt)

nitric acid (1:1 salt)

toluenesulfonic acid (1:1 salt)

methanesulfonic acid (1:1 salt)

p-xylenesulfonic acid (1:1 salt)

2-mesitylenesulfonic acid (1:1 salt)

1,5-naphthalenesulfonic acid (1:0.5 salt)

naphthalenesulfonic acid (1:1 salt)

benzenesulfonic acid (1:1 salt)

saccharinic acid (1:1 salt)

maleic acid (1:1 salt)

phosphoric acid (1:1 salt)

D-glutamic acid (1:1 salt)

L-glutamic acid (1:1 salt)

D,L-glutamic acid (1:1 salt)

L-arginine (1:1 salt)

L-lysine (1:1 salt)

L-lysine hydrochloride (1:1 salt)

glycine (1:1 salt)

salicylic acid (1:1 salt)

tartaric acid (1:1 salt)

fumaric acid (1:1 salt)

citric acid (1:1 salt)

L-(-)-malic acid (1:1 salt)

D,L-malic acid (1:1 salt)

D-gluconic acid (1:1 salt)

Method 3

Preparation of Amorphous Compound A, ethanesulfonic acid salt

Compound A (203 mg; see Preparation A above) was dissolved in ethanol (3 mL) and ethanesulfonic acid (1 eq., 95%, 35 TL) was added to the solution. The mixture was stirred for a few minutes, and then the solvent was evaporated. The resulting oil was slurried in *iso*-octane and evaporated to dryness until a solid material was obtained. Finally, the substance was re-slurried in *iso*-octane and the solvent evaporated again resulting in a white, dry, amorphous solid. The substance was vacuum dried at 40°C overnight.

Methods 4 to 9

Preparation of Crystalline Compound A, ethanesulfonic acid salt

Method 4

Crystallisation of Amorphous Material

Amorphous Compound A, ethanesulfonic acid salt (17.8 mg; see Method 3 above) was slurried in methyl *iso*-butyl ketone (600 TL). After 1 week, crystalline needles were observed, which were filtered off and air-dried.

Methods 5 to 7

Reaction Crystallisations (without Anti-solvent)

Method 5

Compound A (277 mg; see Preparation A above) was dissolved in methyl *iso*-butyl ketone (3.1 mL). Ethanesulfonic acid was added (1 eq., 95%, 48 TL). Precipitation of amorphous ethanesulfonate salt occurred immediately. More methyl *iso*-butyl ketone (6 mL) was added and the slurry was treated with ultrasound. Finally, a third portion of methyl *iso*-butyl ketone (3.6 mL) was added and then the slurry was left overnight with stirring (magnetic stirrer). The next day, the substance had transformed into crystalline needles. The slurry was filtered off, washed with methyl *iso*-butyl ketone (0.5 mL) and air dried.

Method 6

Compound A (236 mg; see Preparation A above) was dissolved at room temperature in methyl *iso*-butyl ketone (7 mL). Ethanesulfonic acid (1 eq., 41TL) was mixed with 2 mL of methyl *iso*-butyl ketone in a vial. The solution of Compound A was seeded with crystalline Compound A, ethanesulfonic acid salt (see Methods 4 and 5 above). Then, 250 TL of the methyl *iso*-butyl ketone solution of ethanesulfonic acid was added in portions over 45 minutes. The solution was seeded again, and the temperature was increased to 30°C. Then, 500 TL of the methyl *iso*-butyl ketone solution was added over approximately 1 hour. The resulting slurry was left overnight before a final amount of the methyl *iso*-butyl ketone/acid solution was added over 20 minutes. The vial was rinsed with 1.5 mL of methyl *iso*-butyl ketone, which was added to the slurry. After a further 6 hours, the crystals were filtered off, washed with methyl *iso*-butyl ketone (2 mL) and dried under reduced pressure at 40°C. A total of 258 mg of crystalline salt was obtained which corresponds to a yield of approximately 87%.

Method 7

Compound A (2.36 g; see Preparation A above) was dissolved in methyl *iso*-butyl ketone (90 mL). Seed crystals (10 mg) of Compound A, ethanesulfonic acid salt (see Methods 4 to 6 above) were added to the solution, and then ethanesulfonic

acid (40 TL) was added in two portions. Further seed crystals (12 mg) and two portions of ethanesulfonic acid (2 x 20 TL) were then added. The slurry was diluted with methyl *iso*-butyl ketone (15 mL) before the addition of ethanesulfonic acid was continued. A total amount of 330 TL ethanesulfonic acid was added, in portions, over 1 hour. A small amount of seed crystals was added and, finally, the slurry was left overnight with stirring. The next day, the crystals were filtered off, washed with methyl *iso*-butyl ketone (2 x 6 mL) and dried under reduced pressure at 40°C. After drying, a total of 2.57 g of white, crystalline product was obtained corresponding to a yield of 89%.

Methods 8 and 9

Reaction Crystallizations (with Anti-solvent)

Method 8

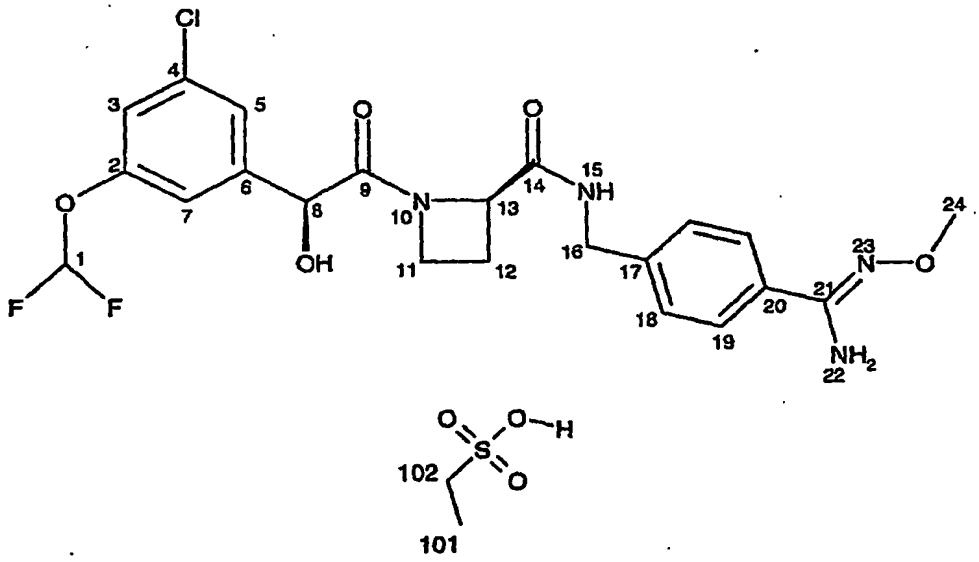
Compound A (163 mg; see Preparation A above) was dissolved in *iso*-propanol (1.2 mL). The solution was heated to 35°C. Ethanesulfonic acid was added (28 TL). Then, ethyl acetate (4.8 mL) was added and the solution was seeded with crystalline Compound A, ethanesulphonic acid salt (see Methods 4 to 7 above). Crystallization started almost immediately. The slurry was left for about 80 minutes at 35°C before being allowed to cool to ambient temperature (21°C). Two hours later, the crystals were filtered off, washed three times with ethyl acetate (3 x 0.4 mL), and dried under reduced pressure at 40°C. A total of 170 mg of crystalline title product was obtained which corresponds to a yield of approximately 82%.

Method 9

Compound A (20.0 g; see Preparation A above) was dissolved in *iso*-propanol (146.6 mL) at 40°C and ethanesulfonic acid (3.46 mL, 95%, 1 eq.) was added to the solution. To the resulting clear solution, seed crystals of Compound A, ethanesulfonic acid salt were added (50 mg; see Methods 4 to 8 above). Then,

ethyl acetate (234 mL) was added over 10 minutes. The resulting slightly opaque solution was seeded once more (70 mg) and left for one hour at 40°C with stirring to allow for crystallization to start. After this, a total of 352 mL of ethyl acetate was added at a constant rate over one hour. When all of the ethyl acetate had been added, the slurry was left for 1 hour, before being cooled to 21°C over 2 hours. The crystallization was allowed to continue for 1 hour at 21°C before the crystals were filtered off, washed twice with ethyl acetate (50 mL + 60 mL) and finally, dried under reduced pressure at 40°C overnight. A total of 21.6 g of a white, crystalline salt was obtained, corresponding to a yield of approximately 90%.

Compound A, ethanesulfonic acid salt is represented below.



Method 10

Preparation of Amorphous Compound A, benzenesulfonic acid salt

Compound A (199 mg; see Preparation A above) was dissolved in ethanol (2 mL). Benzenesulfonic acid (1 eq. 90%, 70mg) was dissolved in ethanol (1 mL) in a vial. The ethanol solution of the acid was added to the solution of Compound A and the vial was rinsed with 1 mL ethanol, which was then added to the mixture. The

mixture was stirred for a few minutes, and then the ethanol was evaporated until an oil was formed. Ethyl acetate (3 mL) was added and the solvent was evaporated again to dryness. An amorphous solid was formed.

Methods 11 to 13

Preparation of Crystalline Compound A, benzenesulfonic acid salt

Method 11

Crystallisation of Amorphous Material

Amorphous Compound A benzenesulfonic acid salt (20.7 mg; see Method 10 above) was slurried in ethyl acetate (600 TL). After 5 days, crystalline needles were observed in the slurry.

Methods 12 and 13

Reaction Crystallisations

Method 12

Compound A (128 mg; see Preparation A above) was dissolved in ethyl acetate (3 mL). The solution was seeded with the slurry from Method 11 above. Then, benzenesulfonic acid was added (1 eq., 90%, 45 mg). Precipitation of benzenesulphonic acid salt occurred immediately. *iso*-Propanol was added to the slurry (0.8 mL) and the mixture was seeded again. Two days later, the substance had transformed into crystalline needles. The slurry was filtered off, washed with ethyl acetate (3 x 0.2 mL) and dried for a short time under vacuum at 40°C. A total of approximately 140 mg of white solid was obtained.

Method 13

Compound A (246 mg; see Preparation A above) was dissolved in *iso*-propanol (1.52 mL). Benzenesulfonic acid was added (88 mg, 90%). To the clear solution, ethyl acetate was added (3 mL), and then the mixture was seeded to initiate

crystallisation. After 1 hour, more ethyl acetate was added (2.77 mL). Finally, the slurry was allowed to crystallise overnight before the crystals were filtered off, washed with ethyl acetate (3 x 0.3 mL) and dried at 40°C under vacuum. A total of 279 mg salt was obtained which corresponds to a yield of approximately 86%.

Method 14

Preparation of Amorphous Compound A, *n*-propanesulfonic acid salt

Compound A (186 mg; see Preparation A above) was dissolved in *iso*-propanol (1.39 mL) and *n*-propanesulfonic acid (1 eq., 95%, 39 TL) was added. Ethyl acetate (5.6 mL) was added and the solvent was evaporated until a dry, amorphous solid was formed.

Methods 15 and 16

Preparation of Crystalline Compound A, *n*-propanesulfonic acid salt

Method 15

Crystallisation of Amorphous Material

Amorphous Compound A, *n*-propanesulfonic acid salt (20 mg; see Method 14 above) was dissolved in *iso*-propanol (60 TL) and *iso*-propyl acetate (180 TL) was added. After three days crystalline needles were observed.

Method 16

Reaction Crystallisation

Compound A (229 mg; see Preparation A above) was dissolved in *iso*-propanol (1.43 mL). *n*-Propanesulfonic acid was added (1 eq., 95%, 48 TL). Ethyl acetate was added (2 mL), and then the solution was seeded with crystalline salt from Method 15 above. Further ethyl acetate was added (5 mL) and the slurry was left overnight to crystallize. The crystals were filtered off, washed with ethyl acetate (3 x 0.3 mL) and dried under vacuum at 40°C.

Abbreviations

Ac	=	acetyl
APCI	=	atmospheric pressure chemical ionisation (in relation to MS)
API	=	atmospheric pressure ionisation (in relation to MS)
aq.	=	aqueous
Aze(& (*S*)-Aze)	=	(*S*)-azetidine-2-carboxylate (unless otherwise specified)
Boc	=	*tert*-butyloxycarbonyl
br	=	broad (in relation to NMR)
CI	=	chemical ionisation (in relation to MS)
d	=	day(s)
d	=	doublet (in relation to NMR)
DCC	=	dicyclohexyl carbodiimide
dd	=	doublet of doublets (in relation to NMR)
DIBAL-H	=	di-isobutylaluminium hydride
DIPEA	=	diisopropylethylamine
DMAP	=	4-(*N*,*N*-dimethyl amino) pyridine
DMF	=	*N*,*N*-dimethylformamide
DMSO	=	dimethylsulfoxide
DSC	=	differential scanning colorimetry
DVT	=	deep vein thrombosis
EDC	=	1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride
eq.	=	equivalents
ES	=	electrospray
ESI	=	electrospray interface
Et	=	ethyl
ether	=	diethyl ether
EtOAc	=	ethyl acetate

	EtOH	=	ethanol
	Et_2O	=	diethyl ether
	HATU	=	*O*-(azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate
	HBTU	=	[*N*,*N*,*N*',*N*'-tetramethyl-*O*-(benzotriazol-1-yl)uronium hexafluorophosphate]
	HCl	=	hydrochloric acid, hydrogen chloride gas or hydrochloride salt (depending on context)
	Hex	=	hexanes
	HOAc	=	acetic acid
	HPLC	=	high performance liquid chromatography
	LC	=	liquid chromatography
	m	=	multiplet (in relation to NMR)
	Me	=	methyl
	MeOH	=	methanol
	min.	=	minute(s)
	MS	=	mass spectroscopy
	MTBE	=	methyl *tert*-butyl ether
	NMR	=	nuclear magnetic resonance
	OAc	=	acetate
	Pab	=	*para*-amidinobenzylamino
	H-Pab	=	*para*-amidinobenzylamine
	Pd/C	=	palladium on carbon
	Ph	=	phenyl
	PyBOP	=	(benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate
	q	=	quartet (in relation to NMR)
	QF	=	tetrabutylammonium fluoride
	rt/RT	=	room temperature
	s	=	singlet (in relation to NMR)

solutol	=	PEG 660 12-hydroxy stearate (a non-ionic surfactant)
t	=	triplet (in relation to NMR)
TBTU	=	[*N,N,N',N'*-tetramethyl-*O*-(benzotriazol-1-yl)uronium tetrafluoroborate]
TEA	=	triethylamine
Teoc	=	2-(trimethylsilyl)ethoxycarbonyl
TEMPO	=	2,2,6,6-tetramethyl-1-piperidinyloxy free radical
TFA	=	trifluoroacetic acid
TGA	=	thermogravimetric analysis
THF	=	tetrahydrofuran
TLC	=	thin layer chromatography
UV	=	ultraviolet

Prefixes *n-*, *s-*, *i-*, *t-* and *tert-* have their usual meanings: normal, secondary, *iso*, and tertiary.

The invention is illustrated by way of the following Examples.

Example 1

Compound A	30 µmol
PEG 400/ethanol/water 50/5/45 (w/w) %	to 1 mL

A formulation was prepared by dissolving Compound A in PEG 400/ethanol/water 50/5/45 (w/w) % followed by gently stirring. This composition was given to dogs orally by gavage once daily for 5 days. The dose 150 µmol/kg gave maximum plasma concentrations in the range 118-254 µM (118-254 µmol/L) after the first dose and 186-286 µM (186-286 µmol/L) after the fifth dose.

Example 2

Compound A	40 µmol

PEG 400/ethanol/water 50/5/45 (w/w) %	to 1 mL

A formulation was prepared by dissolving Compound A in PEG 400/ethanol/water 50/5/45 (w/w) % followed by gently stirring. This composition was given to rats orally by gavage once daily for 5 days. The dose 400 µmol/kg gave maximum plasma concentrations in the range 3.17-6.91 µM (3.17-6.91 µmol/L) after the first dose and 3.01-10.5 µM (3.01-10.5 µmol/L) after the fifth dose.

Example 3

Compound A	80 µmol
PEG 400/ethanol/water 50/5/45 (w/w) %	to 1 mL

A formulation was prepared by dissolving Compound A in PEG 400/ethanol/water 50/5/45 (w/w) % followed by gently stirring. This composition was given to rats orally by gavage once daily for 5 days. The dose 800 µmol/kg gave maximum plasma concentrations in the range 7.00-23.9 µM (7.00-23.9 µmol/L) after the first dose and 10.3-32.8 µM (10.3-32.8 µmol/L) after the fifth dose.

Example 4

Compound A	250 µmol
PEG 400/ethanol/water 50/5/45 (w/w) %	to 1 mL

A formulation was prepared by dissolving Compound A in PEG 400/ethanol/water 50/5/45 (w/w) % followed by gently stirring. The solubility of Compound A is at least 1000 times higher in this vehicle compared to water alone.

Example 5

Compound A	21 µmol
PEG 400/ethanol/water 20/10/70 (w/w) %	to 1 mL

A formulation was prepared by dissolving Compound A in PEG 400/ethanol/water 20/10/70 (w/w) % followed by gently stirring. The solubility of Compound A is at least 100 times higher in this vehicle compared to water alone.

Example 6

Compound A	51 µmol
PEG 400/ethanol/water 20/10/70 (w/w) %	to 1 mL
The water contained 50 µmol/mL Tartaric Acid	

A formulation was prepared by dissolving Compound A in acidified PEG 400/ethanol/water 20/10/70 (w/w) % that was followed by gently stirring. The pH of this solution was 3.6. The solubility of Compound A is at least 250 times higher in this vehicle compared to water alone.

Example 7

Compound A	44 µmol
PEG 400/ethanol/water 30/5/65 (w/w) %	to 1 mL

A formulation was prepared by dissolving Compound A in PEG 400/ethanol/water 30/5/65 (w/w) % followed by gently stirring. The solubility of Compound A is at least 200 times higher in this vehicle compared to water alone.

Example 8

Compound A	88 µmol
PEG 400/ethanol/water 30/5/65 (w/w) %	to 1 mL
The water contained 50 µmol/mL Tartaric Acid	
HCl to pH 3.6	q.s.

A formulation was prepared by dissolving Compound A in acidified PEG 400/ethanol/water 30/5/65 (w/w) % followed by gently stirring. The pH of this solution

was set to 3.6 by addition of HCl. The solubility of Compound A is at least 400 times higher in this vehicle compared to water alone.

Example 9

Compound A	120 µmol
PEG 400/ethanol/water 40/5/55 (w/w) %	to 1 mL

A formulation was prepared by dissolving Compound A in PEG 400/ethanol/water 40/5/55 (w/w) % followed by gently stirring. The solubility of Compound A is at least 600 times higher in this vehicle compared to water alone.

Example 10

Compound A	198 µmol
PEG 400/ethanol/water 40/5/55 (w/w) %	to 1 mL
The water contained 50 µmol/mL Tartaric Acid	
HCl to pH 3.8	q.s.

A formulation was prepared by dissolving Compound A in acidified PEG 400/ethanol/water 40/5/55 (w/w) % followed by gently stirring. The pH of this solution was set to 3.8 by addition of HCl. The solubility of Compound A is at least 1000 times higher in this vehicle compared to water alone.

Example 11

Compound A	136 µmol
Hydroxypropyl-β-cyclodextrin/water 40/60 (w/w) %	to 1 mL
HCl to pH 3.7	q.s.

A formulation was prepared by dissolving Compound A in Hydroxypropyl-β-cyclodextrin/water 40/60 (w/w) % followed by gently stirring. The pH of this solution was

set to 4.7 by addition of HCl. The solubility of Compound A is at least 700 times higher in this vehicle compared to water alone.

Example 12

Compound A	76 µmol
Hydroxypropyl-β-cyclodextrin/water 28/72 (w/w) %	to 1 mL

A formulation was prepared by dissolving Compound A in Hydroxypropyl-β-cyclodextrin/water 28/72 (w/w) % followed by gently stirring. The solubility of Compound A is at least 400 times higher in this vehicle compared to water alone.

Example 13

Compound A	40 µmol
PEG 400/ethanol/solutol™/water 50/5/5/40 (w/w) %	to 1 mL

A formulation was prepared by dissolving Compound A in PEG 400/ethanol/ solutol™/water 50/5/5/40 (w/w) % followed by gently stirring. The solubility of Compound A is at least 80 times higher in this vehicle compared to water alone.

Example 14

Compound A	40 µmol
PEG 400/water 40/60 (w/w) %	to 1 mL

A formulation was prepared by dissolving Compound A in PEG 400 followed by gently stirring for at least 1 hour, thereafter water was added to the final volume. The solubility of Compound A is at least 200 times higher in this vehicle compared to water alone.

Example 15

Compound A	52 µmol
PEG 400/water 35/65 (w/w) %	to 1 mL

The water contained 50 µmol/mL Tartaric Acid

A formulation was prepared by dissolving Compound A in PEG 400 followed by gently stirring for at least 1 hour, thereafter water was added to the final volume. The solubility of Compound A is at least 250 times higher in this vehicle compared to water alone.

Example 16

Compound A	58 µmol
PEG 400/water 50/50 (w/w) %	to 1 mL

A formulation was prepared by dissolving Compound A in PEG 400 followed by gently stirring for at least 1 hour, thereafter water was added to the final volume. The solubility of Compound A is at least 300 times higher in this vehicle compared to water alone.

Example 17

Compound A	88 µmol
PEG 400/water 67/33 (w/w) %	to 1 mL

A formulation was prepared by dissolving Compound A in PEG 400 followed by gently stirring for at least 1 hour, thereafter water was added to the final volume. The solubility of Compound A is at least 400 times higher in this vehicle compared to water alone.

Example 18

Compound A	92 µmol
PEG 400/ethanol/water 45/1/54 (w/w) %	to 1 mL

A formulation was prepared by dissolving Compound A in PEG 400/ethanol/water 45/1/54 (w/w) % followed by gently stirring. The solubility of Compound A is at least 450 times higher in this vehicle compared to water alone.

Example 19

Compound A	159 µmol
PEG 400/ethanol/water 45/1/54 (w/w) %	to 1 mL
The water contained 50 µmol/mL Tartaric Acid	
HCl to pH 4.2	q.s.

A formulation was prepared by dissolving Compound A in acidified PEG 400/ethanol/water 45/1/54 (w/w) % followed by gently stirring. The pH of this solution was set to 4.2 with HCl. The solubility of Compound A is at least 800 times higher in this vehicle compared to water alone.

Example 20

Compound A	101 µmol
PEG 400/ethanol/water 45/2/53 (w/w) %	to 1 mL

A formulation was prepared by dissolving Compound A in PEG 400/ethanol/water 45/2/53 (w/w) % followed by gently stirring. The solubility of Compound A is at least 500 times higher in this vehicle compared to water alone.

Example 21

Compound A	167 µmol
PEG 400/ethanol/water 45/2/53 (w/w) %	to 1 mL
The water contained 50 µmol/mL Tartaric Acid	
HCl to pH 4.3	q.s.

A formulation was prepared by dissolving Compound A in acidified PEG 400/ethanol/water 45/2/53 (w/w) %followed by gently stirring. The pH of this solution was set to 4.3 by addition of HCl. The solubility of Compound A is at least 800 times higher in this vehicle compared to water alone.

Example 22

Compound A	46 µmol
DMA/water 50/50 (w/w) %	to 1 mL

A formulation was prepared by dissolving Compound A in the vehicle followed by gently stirring for at least 1 hour. The solubility of Compound A is at least 230 times higher in this vehicle compared to water alone.

Example 23

Compound A	29 µmol
DMA/water 25/75 (w/w) %	to 1 mL

A formulation was prepared by dissolving Compound A in the vehicle followed by gently stirring for at least 1 hour. The solubility of Compound A is at least 150 times higher in this vehicle compared to water alone.

Example 24

Compound A	5 µmol
HCl	10 µmol
Water	to 1 mL
HCl/NaOH to pH 3.6	q.s.

A formulation was prepared by dissolving Compound A in a lower volume of the double equimolar amount of HCl followed by gently stirring and dilution to 1mL. The pH of the final solution was adjusted to 3.6. The solubility of Compound A is at least 20 times higher in this vehicle compared to water alone.

Example 25

Compound A	10 µmol
Water	to 1 mL

HCl to pH 1.0	q.s.
NaOH to pH 3.0	q.s.

A formulation was prepared by dissolving Compound A water and HCl was added to give pH 1 thereafter the solution was gently stirred. The pH of the final solution was adjusted to 3.0 with NaOH. The solubility of Compound A is at least 40 times higher in this vehicle compared to water alone. This formulation was given p.o to rats in a kinetic comparative study.

Example 26

Compound A	100 µmol
Miglyol	0.25 g/g Compound A
DMA	to 1 mL

A formulation was prepared by dissolving Compound A in 1mL DMA/miglyol followed by gently stirring. The solubility of Compound A is at least 4000 times higher in this vehicle compared to water alone.

Example 27

Compound A	100 µmol
Miglyol	0.25 g/g Compound A
Ethanol	to 1 mL

A formulation was prepared by dissolving Compound A in 1mL Ethanol/Miglyol followed by gently stirring. The solubility of Compound A is at least 4000 times higher in this vehicle compared to water alone.

Example 28

Compound A	130 µmol
Ethanol	to 1 mL

A formulation was prepared by dissolving Compound A in 1mL ethanol followed by gently stirring. The substance is stable in this formulation more than 1 week.

Example 29

In order to prepare nanoparticles a stock solution of Compound A of about 100 mM in ethanol was used. Included was also 25% (w/w) Miglyol, calculated on the amount of the substance. The solutions were diluted 1/10 with the stabilizer solution, consisting of 0.2% (w/w) PVP and 0.25 mM SDS in water. The mixing, which is considered as a critical parameter during the nanoparticle preparation, was rapid and instant. The drug solution was rapidly injected into the stabilizer solution during ultrasonication. After the 1/10 dilution in the aqeous solution, nanoparticles of about 150 nm were achieved. After 6 hours at room temperature, the particle sizes were unchanged.

Example 30

Compound A	4 µmol
saline/ethanol/solutol 90/5/5 (w/w) %	to 1 mL

A formulation was prepared by dissolving Compound A in saline/ethanol/solutol 90/5/5 (w/w) % followed by gently stirring. The solution was given orally to rats and the plasma concentration Compound D was 0.56 µmol/L after 1 hour. The solution was given orally to rats and the plasma concentration of Compound D was 0.56 µmol/L after 1 hour. The solution was given subcutaneously to rats and the plasma concentration of Compound D and A were

Example 31

Compound B	4 µmol
saline/ethanol/solutol 90/5/5 (w/w) %	to 1 mL

A formulation was prepared by dissolving Compound B in saline/ethanol/solutol 90/5/5 (w/w) % followed by gently stirring. The solution was given orally to rats and the plasma concentration Compound B and Compound E were 0.07 µmol/L and 0.65 µmol/L, after 1 hour. The solution was given subcutaneously to rats and the plasma concentration of Compound B and E were 0.07 µmol/L and 0.65 µmol/L, respectively, after 1 hour.

Example 32

Compound C	4 µmol
saline/ethanol/solutol 90/5/5 (w/w) %	to 1 mL

A formulation was prepared by dissolving Compound C in saline/ethanol/solutol 90/5/5 (w/w) % followed by gently stirring. The solution was given orally to rats and the plasma concentration of Compound C and F were 0.2 µmol/L and 0.5 µmol/L after 1 hour. The solution was given subcutaneously to rats and the plasma concentration of Compound C and F were 0.35 µmol/L and 0.5 µmol/L, respectively, after 1 hour.

Example 33

Compound D (trifluoroacetate salt)	5 µmol
Saline 9 mg/ml	to 1 mL

A formulation was prepared by dissolving the salt of Compound D in 1mL saline followed by gently stirring.

Example 34

Compound D (trifluoroacetate salt)	75 µmol
EtOH	0.05 mL
Saline(9 mg/ml)	to 1 mL

A formulation was prepared by dissolving the salt of Compound D in 1mL saline/ethanol solution followed by gently stirring.

Example 35

Compound D (trifluoroacetate salt)	4 µmol
EtOH	0.02 mL
saline	to 1 mL

A formulation was prepared by dissolving the salt of Compound D in 1mL saline/etanol solution followed by gently stirring. The solution was given subcutaneously to rats and the plasma concentration of Compound D was 0.55 µmol/L

Example 36

Compound E (acetate salt)	4 µmol
EtOH	0.02 mL
saline	to 1 mL

A formulation was prepared by dissolving the salt of Compound E in 1mL saline/ethanol solution followed by gently stirring. The solution was given subcutaneously to rats and the plasma concentration of Compound E was 0.75 µmol/L after

Example 37

Compound F (trifluoroacetate salt)	4 µmol
EtOH	0.02 mL
saline	to 1 mL

A formulation was prepared by dissolving the salt of Compound F in 1mL saline/ethanol solution followed by gently stirring. The solution was given subcutaneously to rats and the plasma concentration Compound F was 0.92 µmol/L after 1 hour.

Example 38

Compound E (acetate salt)	22 mg

Saline 9 mg/ml	to 1 mL

A formulation was prepared by dissolving the salt of Compound E in 1mL saline followed by gently stirring.

Example 39

Compound F (trifluoroacetate salt)	22 mg
Saline 9 mg/ml	to 1 mL

A formulation was prepared by dissolving the salt of Compound F in 1mL saline followed by gently stirring.

Example 40

Compound A (as esylate salt)	14 mg
water	to 1 mL

A solution was prepared by dissolving excess of Compound A as esylate salt in 3mL water followed by gently stirring over night. A final concentration of the solution after filtration was monitored to 14 mg/ml at a pH of 2.7.

Example 41

Compound A (as esylate salt)	33 mg
Sodium phosphate buffer pH=3.1 I=0.1	to 1 mL

A solution was prepared by dissolving 112 g of Compound A as esylate salt in 3mL sodium phosphate buffer followed by gently stirring over night. A final concentration of the solution after filtration was monitored to 33 mg/ml at a pH of 2.7.

Example 42

Compound A (as esylate salt)	1.6 mg

Sodium phosphate buffer pH=6.9 I=0.1	to 1 mL

A solution was prepared by dissolving 20 mg of Compound A as esylate salt in 3mL sodium phosphate buffer followed by gently stirring over night. A final concentration of the solution after filtration was monitored to 1.6 mg/ml at a pH of 6.5.

Example 43

The following freeze dried formulations can be made in accordance with techniques described in one or more of Examples 1-29 above:

a.

Compound A	10 µmol
Mannitol	10 mg
Water	to 1 mL
HCl to pH 1.0	q.s.
NaOH to pH 3.0	q.s.

b.

Compound D	10 µmol
Mannitol	10 mg
Water	to 1 mL
HCl to pH 1.0	q.s.
NaOH to pH 3.0	q.s.

c.

Compound E	10 µmol
Mannitol	10 mg
Water	to 1 mL
HCl to pH 1.0	q.s.
NaOH to pH 3.0	q.s.

d.	
Compound F	10 µmol
Mannitol	10 mg
Water	to 1 mL
HCl to pH 1.0	q.s.
NaOH to pH 3.0	q.s.
e.	
Compound B	10 µmol
Mannitol	10 mg
Water	to 1 mL
HCl to pH 1.0	q.s.
NaOH to pH 3.0	q.s.
g.	
Compound C	10 µmol
Mannitol	10 mg
Water	to 1 mL
HCl to pH 1.0	q.s.
NaOH to pH 3.0	q.s.
g.	
Compound A (as esylate salt)	14 mg
Mannitol	10 mg
Water	to 1 mL
HCl to pH 1.0	q.s.
NaOH to pH 3.0	q.s.

The solutions are optionally sterile filtered, for example trough a 0.22 µm membrane filter. Solutions (sterile or otherwise) are filled into appropriate vessels (e. g. vials) and the

formulations are freeze-died using standard equipment. Vials may be sealed in freeze-dryer equipment under nitrogen atmosphere.

Example 44

	Weight	Amount
Compound A	48 mg	17%
Polyvinyl pyrrolidone K90	8 mg	3%
Mannitol	21 mg	7%
Microcrystalline cellulose	187 mg	65%
Sodium starch glycolate	21 mg	7%
Sodium stearyl fumarate	3 mg	1%

The excipients and drug were mixed and granulated with polyvinyl pyrrolidone K90 dissolved in water. The granules were then dried in a drying oven. The granulate was lubricated with sodiumstearylfumarate and compressed into tablets using an exenterpress.

Three individual tablets were tested for drug release in 900ml media using a USP dissolution apparatus 2 (paddle+basket[1]) at 50 rpm and 37°C. The dissolution media used were 0.1 M hydrochloric acid (pH 1) and 0.1 M sodium phosphate buffer (pH 6.8). In-line quantitation was performed using the C Technologies fibre optic system with 220 nm as the analytical wavelength when 0.1 M HCl was used as the dissolution media and with 260 nm as the analytical wavelength when phosphate buffer pH 6.8 was used as the dissolution media. 350 nm was used as the reference wavelength with both media. For the first two hours of the analysis the release value was measured every 15 minutes, and then every hour for the remainder of the analysis. The results are presented in the table below.

[[1] A custom made quadrangular basket of mesh wire, soldered in one of its upper, narrow sides to the end of a steel rod. The rod is brought through the cover of the dissolution vessel and fixed by means of two Teflon nuts, 3.2cm from the centre of the vessel. The lower edge of the bottom of the basket is adjusted to be 1cm above the paddle. The basket is directed along the flow stream with the tablet under test standing on its edge.]

Time (min)	% released in buffer pH 1.1	% released in buffer pH 6.8
0	0	0
15	100	44
30	100	49
45	100	51
60	100	53
120	100	57
180	100	61
240	100	63
360	100	67
480	100	70
600	100	75
720	100	77
840	100	79
960	100	82
1080	100	83
1200	100	86

Example 45

	Weight	Amount
Esylate salt of Compound A	58 mg	20%
Polyvinyl pyrrolidone K90	8 mg	3%
Mannitol	21 mg	7%
Microcrystalline cellulose	177 mg	62%
Sodium starch glycolate	21 mg	7%
Sodium stearyl fumarate	3 mg	1%

The excipients and drug were mixed and granulated with polyvinyl pyrrolidone K90 dissolved in water. The granules were then dried in a drying

oven. The granulate was lubricated with sodium stearyl fumarate and compressed into tablets using an exenterpress.

Example 46

	Weight	Amount
Compound B	48 mg	17%
Polyvinyl pyrrolidone K90	8 mg	3%
Mannitol	21 mg	7%
Microcrystalline cellulose	187 mg	65%
Sodium starch glycolate	21 mg	7%
Sodium stearyl fumarate	3 mg	1%

The excipients and drug were mixed and granulated with polyvinyl pyrrolidone K90 dissolved in water. The granules were then dried in a drying oven. The granulate was lubricated with sodium stearyl fumarate and compressed into tablets using an exenterpress

Example 47

	Weight	Amount
Compound C	48 mg	17%
Polyvinyl pyrrolidone K90	8 mg	3%
Mannitol	21 mg	7%
Microcrystalline cellulose	187 mg	65%
Sodium starch glycolate	21 mg	7%
Sodium stearyl fumarate	3 mg	1%

The excipients and drug were mixed and granulated with polyvinyl pyrrolidone K90 dissolved in water. The granules were then dried in a drying

oven. The granulate was lubricated with sodium stearyl fumarate and compressed into tablets using an exenterpress

Example 48

Compound A	16 µmol
PEG 414	to 1 mL

A formulation was prepared by dissolving Compound A in acidified PEG414 followed by gently stirring.

Example 49

Compound A	16 µmol
PEG 300	to 1 mL

A formulation was prepared by dissolving Compound A in acidified PEG300 followed by gently stirring.

Example 50

Compound A	16 µmol
PEG 200	to 1 mL

A formulation was prepared by dissolving Compound A in acidified PEG200 followed by gently stirring.

Example 51

Compound G	4 µmol
saline/ethanol/solutol 90/5/5 (w/w) %	to 1 mL

A formulation was prepared by dissolving Compound G in saline/ethanol/solutol 90/5/5 (w/w) % followed by gently stirring.

Example 52

Compound J	4 µmol
saline/ethanol/solutol 90/5/5 (w/w) %	to 1 mL

A formulation was prepared by dissolving Compound J in saline/ethanol/solutol 90/5/5 (w/w) % followed by gently stirring.

Example 53

Compound H	4µmol
saline/ethanol/solutol 90/5/5 (w/w) %	to 1 mL

A formulation was prepared by dissolving Compound H in saline/ethanol/solutol 90/5/5 (w/w) % followed by gently stirring.

Example 54

	Weight	Amount
Compound A esylate salt	500 mg	66%
Polyvinyl pyrrolidone K30	100 mg	13%
Microcrystalline cellulose	100 mg	13%
Crosslinked sodium CMC	50 mg	7%
Magnesium stearate	.5 mg	1%

Formulation can be prepared in accordance with Example 47 above.

Example 55

	Weight	Amount
Compound A *n*-propane sulphonic acid salt	100 mg	23%
Polyvinyl pyrrolidone K30	60 mg	14%
Lactose monohydrate	100 mg	23%
Microcrystalline cellulose	150 mg	34%
Polyvinyl pyrrolidone crosslinked	20 mg	5%
Sodium stearyl fumarate	10 mg	2%

Formulation can be prepared in accordance with Example 47 above.

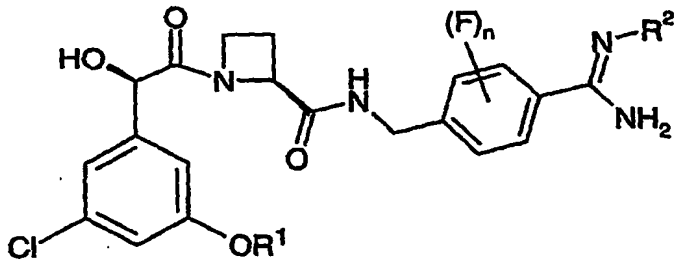
Example 56

	Weight	Amount
Compound A besylate salt	20 mg	8%
Hydroxypropyl cellulose	15 mg	6%
Microcrystalline cellulose	200 mg	79%
Crosslinked sodium CMC	15 mg	6%
Sodium stearyl fumarate	3 mg	1%

Formulation can be prepared in accordance with Example 47 above.

CLAIMS

1. An immediate release pharmaceutical formulation comprising, as active ingredient, a compound of formula (I):



(I)

wherein

R^1 represents C_{1-2} alkyl substituted by one or more fluoro substituents;

R^2 represents hydrogen, hydroxy, methoxy or ethoxy; and

n represents 0, 1 or 2;

or a pharmaceutically acceptable salt thereof; and a pharmaceutically acceptable diluent or carrier;

provided that the formulation does not solely contain:

- a solution of one active ingredient and water;
- a solution of one active ingredient and dimethylsulphoxide; or,
- a solution of one active ingredient in a mixture of ethanol : PEG 660 12-hydroxy stearate : water 5:5:90.

2. An immediate release pharmaceutical formulation as claimed in claim 1 wherein the active ingredient is:

Ph(3-Cl)(5-$OCHF_2$)-(*R*)CH(OH)C(O)-(*S*)Aze-Pab(OMe);

Ph(3-Cl)(5-$OCHF_2$)-(*R*)CH(OH)C(O)-(*S*)Aze-Pab(2,6-diF)(OMe);

Ph(3-Cl)(5-OCH_2CH_2F)-(*R*)CH(OH)C(O)-(*S*)Aze-Pab(OMe);

Ph(3-Cl)(5-$OCHF_2$)-(*R*)CH(OH)C(O)-(*S*)Aze-Pab;

Ph(3-Cl)(5-$OCHF_2$)-(*R*)CH(OH)C(O)-(*S*)Aze-Pab(OH);

Ph(3-Cl)(5-$OCHF_2$)-(*R*)CH(OH)C(O)-(*S*)Aze-Pab(2,6-diF);

Ph(3-Cl)(5-$OCHF_2$)-(*R*)CH(OH)C(O)-(*S*)Aze-Pab(2,6-diF)(OH);
Ph(3-Cl)(5-OCH_2CH_2F)-(*R*)CH(OH)C(O)-(*S*)Aze-Pab; or,
Ph(3-Cl)(5-OCH_2CH_2F)-(*R*)CH(OH)C(O)-(*S*)Aze-Pab(OH).

3. A solid immediate release pharmaceutical formulation as claimed in claim 1 wherein the active ingredient is:
Ph(3-Cl)(5-$OCHF_2$)-(*R*)CH(OH)C(O)-(*S*)Aze-Pab(OMe);
Ph(3-Cl)(5-$OCHF_2$)-(*R*)CH(OH)C(O)-(*S*)Aze-Pab(2,6-diF)(OMe); or,
Ph(3-Cl)(5-OCH_2CH_2F)-(*R*)CH(OH)C(O)-(*S*)Aze-Pab(OMe),
or a pharmaceutically acceptable salt thereof.

4. A solid immediate release pharmaceutical formulation as claimed in claim 1 wherein the active ingredient is Ph(3-Cl)(5-$OCHF_2$)-(*R*)CH(OH)C(O)-(*S*)Aze-Pab(OMe) or a C_{1-6} alkanesulfonic acid or an optionally substituted arylsulfonic acid salt thereof.

5. An injectable immediate release pharmaceutical formulation as claimed in claim 1 wherein the active ingredient is:
Ph(3-Cl)(5-$OCHF_2$)-(*R*)CH(OH)C(O)-(*S*)Aze-Pab;
Ph(3-Cl)(5-$OCHF_2$)-(*R*)CH(OH)C(O)-(*S*)Aze-Pab(2,6-diF); or
Ph(3-Cl)(5-OCH_2CH_2F)-(*R*)CH(OH)C(O)-(*S*)Aze-Pab.

6. The use of a formulation as claimed in claim 1 as a medicament.

7. The use of a formulation as claimed in claim 1 in the manufacture of a medicament for the treatment of a cardiovascular disorder.

8. A method of treating a cardiovascular disorder in a patient suffering from, or at risk of, said disorder, which comprises administering to the patient a

therapeutically effective amount of a pharmaceutical formulation as claimed in claim 1.

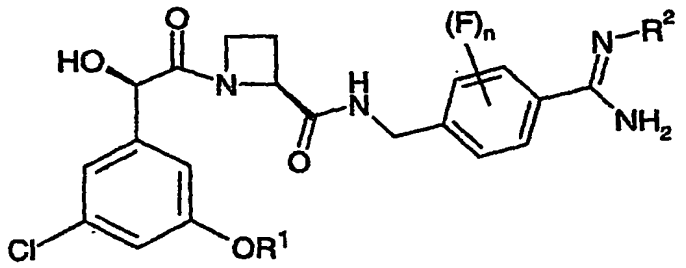
9. A process for making an immediate release formulation as claimed in claim 1.

10. The compound Ph(3-Cl)(5-$OCHF_2$)-(*R*)CH(OH)C(O)-(*S*)Aze-Pab(2,6-diF)(OH).

ABSTRACT

IMMEDIATE RELEASE PHARMACEUTICAL FORMULATION

According to the present invention there is provided an immediate release pharmaceutical formulation comprising, as active ingredient, a compound of formula (I):



(I)

wherein

R^1 represents C_{1-2} alkyl substituted by one or more fluoro substituents;

R^2 represents hydrogen, hydroxy, methoxy or ethoxy; and

n represents 0, 1 or 2;

or a pharmaceutically acceptable salt thereof; and a pharmaceutically acceptable diluent or carrier;

provided that the formulation does not solely contain:

- a solution of one active ingredient and water;
- a solution of one active ingredient and dimethylsulphoxide; or,
- a solution of one active ingredient in a mixture of ethanol : PEG 660 12-hydroxy stearate : water 5:5:90.